# Breast density

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# Breast Density: why all the fuss?

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Title Page

# Breast Density: why all the fuss?

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The term ‘breast density’ or mammographic density (MD) denotes those components of breast parenchyma visualised at mammography that are denser than adipose tissue. MD is composed of a mixture of epithelial and stromal components, notably collagen, in variable proportions.

MD is most commonly assessed in clinical practice with the time-honoured method of visual estimation of area-based percent density (PMD) on a mammogram, with categorisation into quartiles. The computerised semi-automated thresholding method, Cumulus, also yielding area-based percent density, is widely used for research purposes. However, the advent of fully automated volumetric methods developed as a consequence of the widespread use of digital mammography (DM) and yielding both absolute and percent dense volumes, has resulted in an explosion of interest in MD recently.

Broadly, the importance of MD is two-fold: firstly, the presence of marked MD significantly reduces mammographic sensitivity for breast cancer, even with state-of-the-art DM. Recognition of this led to the formation of a powerful lobby group (‘Are You Dense’) in the US, as a consequence of which thirty-two states have legislated for mandatory disclosure of MD to women undergoing mammography. Secondly, it is now widely accepted that MD is in itself a risk factor for breast cancer, with a four to six-fold increased relative risk in women with PMD in the highest quintile compared to those with PMD in the lowest quintile. Consequently, major research efforts are underway to assess whether use of MD could provide a major step forward towards risk-adapted, personalised breast cancer prevention, imaging, and treatment.

## Introduction

The adult female breast is composed of variable proportions of adipose tissue and fibroglandular parenchyma. At mammography, fibroglandular parenchyma, being denser than adipose tissue, attenuates xrays more and appears whiter; hence the term breast density or mammographic density, MD (figure 1). These terms are often used interchangeably and are effectively synonymous. Breast radiologists have always consciously or subconsciously assessed the amount of MD in relation to the visualised total breast area; breasts with little or no MD are regarded as non-dense, whereas breasts in which MD occupies more than 50% of the visualised total breast area are regarded as dense (1).

MD has excited much interest and debate amongst breast clinicians and researchers in the past decade.

There are two main reasons for this: firstly, the association of higher levels of MD with reduced mammographic sensitivity (2–4) and secondly, the positive association of mammographic density with breast cancer risk (5). This is particularly important as MD is modifiable (6–8). A major research effort is underway to elucidate the nature of the risk-conferring components of MD and to understand the underlying genetic, epigenetic and molecular mechanisms involved. Meanwhile, imaging scientists continue to explore methods of assessing MD in a robust and quantifiable fashion, while breast radiologists and epidemiologists devote much effort into establishing the most efficacious approaches to screening and imaging the dense breast. This review will discuss the nature of density, the risks associated with it and how it can be assessed. Finally, it will discuss how density might be incorporated into routine clinical practice in the near future.

## Breast composition

Mammographic density comprises two main components; epithelial or glandular elements and supporting stroma, the latter containing stromal cells, collagen and extracellular matrix (9). Early studies of MD, using tissues obtained from mastectomy, biopsy or autopsy specimens showed variable

increases in the proportions of both epithelial elements (largely reflecting the number of terminal duct lobular units, TDLUs) and stromal components (particularly collagen) and decreases in the proportions of adipose in dense tissues (9,10). Some studies have shown an increase in nuclear and glandular area, findings confirmed in a study in which image guidance was used to biopsy dense and non-dense areas of the same breast in women with no known history of breast disease (11). However, increases in the stromal area were greater still, by up to 11-fold. Other studies have shown differences in glandular complexity and the number of acini per TDLU (12), but these samples were from high risk women. Part of the discrepancy in the literature regarding the histological nature of MD may be attributable to varying risk profiles of the population studied and failure to control for confounding factors.

Attention has also focused on the composition of the extracellular matrix and collagen organisation within breast stroma. Small proteoglycans and stromal matrix regulators such as metalloproteinases are differentially expressed in stroma from high MD breasts (13,14), and recent research has shown that the organisation of collagen fibrils in periductal stroma differs in areas of high and low MD (15). McConnell et al used atomic force microscopy and spectroscopy to show an abundance of large, (>80um) aligned periductal collagen fibrils in high MD tissues, resulting in locally increased stiffness rather than a diffuse increase in fibrosis (15). Second harmonic generation imaging has also demonstrated the presence of more organised stromal collagen in areas of high MD (16). However, the mechanisms behind the altered collagen architecture have yet to be elucidated. **Factors influencing MD**

with age after early adulthood, especially after the menopause (17). The International Consortium on Mammographic Density (ICMD) recently studied differences in MD by age and menopausal status in over 11,000 women with no history of breast cancer from 40 ethnic groups in 22 countries. They showed that MD decreased with age both pre- and postmenopausally, with a large age-adjusted difference in percent MD (PMD) between pre- and postmenopausal women regardless of ethnicity (18).

Life-course body size also has a significant effect on breast density. A high BMI and large breast size are associated with reduced MD; a higher BMI at any age during childhood is associated with lower MD in adulthood. The odds of high MD are increased with later menarche, some studies also finding that greater birthweight and adult height are positively associated with high MD (19). A study from the UK found that first generation South Asian and Afro-Caribbean women had lower age-adjusted breast density than Caucasians, only partly explained by higher BMI and lifestyle factors (20). This group later showed that while BMI, parity and ethnicity were associated with PMD, these factors did not affect the within-woman rate of change of PMD at each age. This high degree of tracking of PMD for a woman with age is important, as it suggests that high PMD could be identified by a single mammographic examination at a young age (21).

Numerous studies have documented the increase in PMD associated with hormone replacement therapy (HRT), specifically combined oestrogen/progestogen therapy (7,8,22) (figure 2). Since HRT is also associated with increased breast cancer incidence, the question arises whether causality is mediated via MD. A recent case-control study within the Women's Health Initiative Trial found that for each 1% increase in PMD one year after commencement of HRT, breast cancer risk increased by 3% (23). For women in the highest quintile of PMD change (>19.3% increase), breast cancer risk increased over 3fold and after adjusting for change in MD, the effect of HRT on breast cancer risk was removed. The implications of this for counselling of women considering HRT are significant.

Lifestyle and reproductive factors probably only account for about 35-40% of the variance in MD; the remaining 60-65% is genetically determined (24–27). Boyd and colleagues undertook two twin studies in Australia and North America, in which they demonstrated that after controlling for relevant covariates, the correlation coefficient for PMD was over 0.6 for monozygotic pairs. At any given age, heritability was

estimated to account for 63% (95% CI 59-75%) of the variation in MD in all twins (24). They later showed that both dense and non-dense areas shared the same high heritability (25).

A number of genome-wide association studies have investigated associations of single nucleotide polymorphisms (SNPs) and MD. These include polymorphisms of genes involved in epidermal growth factors, EGF (*AREG*), oestrogen receptors, ER (*ESR1*), and insulin-like growth factor signalling, as well as cell proliferation, migration (*LSP1*) and tissue vascularisation (28–30). Some of these SNPs also influence breast cancer risk, a recent meta-analysis finding that of 77 known breast cancer susceptibility SNPs, 18% were associated with at least one measure of MD (absolute or percent dense area) (31), lending credence to the suggestion that MD is causally linked with breast cancer.

**How common is MD?**

The prevalence of high MD is highly population-dependent. A study from the US found that around 40% of women aged 40-59 years had more than 50% area based MD, and the corresponding figure for women aged between 60-80 years was 25% (32). Data taken from the Breast Cancer Surveillance Consortium (BCSC) in the US demonstrated heterogeneously or extremely dense breasts in 43% of women aged 40-74 years (33). On the other hand, recent data from an urban screening centre in the UK found that 50% of women had predominantly fatty breasts and only 32%, heterogeneously dense breasts (Dr Louise Wilkinson, personal communication). Urbanisation and degree of social deprivation can result in quite striking regional differences in incidence of MD, even in small countries (34).

**The clinical importance of breast density**

*Reduced mammographic sensitivity*

The recognition that increased MD can reduce mammographic sensitivity is not new. In 1977, Egan and Mosteller found that many more cancers developed within 6 to 36 months after a normal study in dense

breasts compared with fatty breasts (35). They suggested that the increased breast cancer risk described by Wolfe (36) in association with certain parenchymal patterns was in fact attributable to masking of breast cancers in the denser breast.

Subsequently many studies have confirmed the relationship between high MD and reduced mammographic sensitivity (3,4,37–42). This probably accounts for the larger size of screen-detected cancers in dense breasts compared to fatty breasts (43–45) and the excess of interval cancers in dense breasts compared to fatty breasts (3,37,38,40). In one study of over four thousand women (97% of whom were undergoing screening or surveillance mammography), the sensitivity of mammography for impalpable cancers was 80% in non-dense breasts and 56% in dense breasts (40). A nested case-control study within the NHS Breast Screening Programme in East Anglia showed a much higher odds ratio (OR) for interval cancers than screen-detected cancers in P2 and DY groups, especially in the first 18 months after the last screening mammogram (ORs 3.8 and 4.1 respectively) (37). Porter et al later found that screen-detected cancers were significantly smaller in fatty breasts and that there was a statistically significant increase in the proportion of dense breasts in women presenting with interval cancers rather than screen-detected cancers (44).

These early studies all considered screen-film mammography (SFM) but the introduction of full-field digital mammography (DM) has only partly overcome the problem. Though the DMIST study did demonstrate a statistically significantly improved performance for DM over SFM in the dense breast, with areas under the ROC curve of 0.78 and 0.68 respectively, sensitivity was still much lower in dense breasts, a finding confirmed in a subsequent cohort study (2,46).

Data from one Dutch screening unit using DM between 2003 and 2011 has shown screening sensitivity to fall progressively with increasing MD, from 85.7% in fatty breasts to 61% in very dense breasts (47). The authors also found a progressive increase in the number of false positive recalls from screening to

assessment with increasing MD, from 11.2% in predominantly fatty breasts to 23.8% in very dense breasts (47). In a further study, using volumetric density measures, they demonstrated a strong positive association of volumetric MD with interval cancer rates with a hazard ratio (HR) of 8.37 for extremely dense breasts compared to fatty breasts (48).

The phenomenon of reduced mammographic sensitivity in the dense breast has received an enormous amount of publicity, particularly in the United States. The 'Are You Dense' Advocacy group (http://www.areyoudenseadvocacy.org/) was founded by Dr Nancy Capello in 2004, after she was diagnosed with locally advanced breast cancer shortly after a normal screening mammogram. Subsequently Connecticut became the first US state to pass a law mandating the reporting of breast density at mammography and communication of this to the woman. Since then, a further 31 states have passed similar legislation. In six states, the provision of insurance cover for supplemental screening in women with dense breasts is also mandated. The level of information communicated to the woman varies by state and where dense breasts are defined, it is with BI-RADS categories. Defining the population that might benefit from supplemental screening and the best means of doing this is hotly debated (see below).

The influence of MD on mammographic performance also affects symptomatic populations. Numerous studies have demonstrated lower mammographic sensitivity in younger women with dense breasts (4,39,41). A recent study of the performance of DM and US in the diagnosis of cancer in women under 40 found that patients with a false negative mammogram were much more likely to have dense breasts (49) and in a study from 2016 on the predictors of ultrasound-only visible cancers, higher PMD was the strongest predictor for the failure of mammography (50).

Cancers in dense breasts are associated with higher T stages and greater likelihood of lymph node positivity at diagnosis (51). Larger tumour size at diagnosis probably accounts for the poorer prognosis of cancers in dense breast (52,53). In a case-control study of interval and screen-detected cancers, Eriksson

et al found that the HRs for breast cancer survival were three times higher in interval cancers (53), but after adjustment for tumour size (used as a proxy for time to diagnosis), survival differences in dense breasts disappeared. Interestingly, there was still a statistically significantly increased HR for interval cancers in non-dense breasts (5-yr survival HR 2.43, p 0.001), suggesting that interval cancers developing in non-dense breasts are truly biologically more aggressive. This observation has been made elsewhere (54,55), one study finding that interval cancers in non-dense breasts were more likely to be triple negative or HER2 amplified and lymph node positive (54).

*Increased breast cancer risk*

The second reason for the clinical importance of MD is that it is in itself a risk factor for the development of breast cancer. In Wolfe's original paper, he described four xeroradiographic patterns (N1, P1, P2 and DY) where N1 represents the fatty breast, P1 a breast with a nodular pattern of fibroglandular parenchyma involving no more than 25% of the visualised breast area (termed a prominent 'duct pattern'), P2 with a more extensive 'ductal pattern' and DY, a so-called 'dysplastic' breast with uniformly increased MD (36) (Figure 1). Types N1 and P1 are regarded as non-dense and P2 and DY as dense; the latter were both associated with markedly increased breast cancer risk. Subsequently, Wolfe demonstrated a strong correlation between parenchymal patterns and area-based PMD measured with planimetry, finding that the relative risk of breast cancer was even greater for breasts with more than 25% density than it was for the P2 and DY patterns (56).

The question as to whether MD was truly a risk factor or whether the increased incidence could be attributed solely to masking (35,57) has been settled (58). Boyd et al carried out three nested casecontrol studies in three Canadian screening populations with a total of 1,112 matched case-control pairs (58). PMD was categorised visually into sextiles and using a computerised semi-automated thresholding method. After adjustment for confounders, the OR for those with 75% or more MD compared to those

with under 10% was 4.7. Calculation of attributable risk suggested that MD accounted for many breast cancer cases regardless of mode of detection (screen detected or interval). An exceptionally high OR of 17.8 for women with MD ≥75% within one year of a normal screen was secondary to masking, but thereafter the OR remained high at 5.7, suggesting causality. It has been estimated that for every 3-6% increase in MD, relative breast cancer risk increases by 10% (59), and this association has been shown to persist over extended periods (60,61).

A meta-analysis from 2006 included data from more than 14,000 breast cancer cases and 226,000 noncases from 42 studies (5). Higher MD was consistently associated with increased risk despite the great variations in populations studied, method of assessing MD and whether incident or prevalent cancers were considered. Relative risks were much stronger when PMD was assessed, rather than Wolfe patterns, varying from 4 to 6 comparing the least and most dense breasts. An elegant prospective study of women in the Swedish Kopparberg randomised controlled trial looked at the effect of baseline breast density on breast cancer incidence, stage, mortality and screening performance (42). By considering preclinical (screen-detected) and symptomatic (interval) cancers, they were able to confirm higher preclinical incidence rates (indicating causality) but also shorter mean sojourn times (indicating masking) in dense breasts compared with non-dense breasts.

Only age and BRCA carrier status are associated with larger relative risks than extremes of PMD and since high PMD is common, the population attributable risk is also very high. Boyd estimated that for women under the median age of 56 years, the attributable risk for PMD greater than 50% was 26% for all cancers and similar estimates have been obtained in other cohorts (58,62). By contrast, the population attributable risk for BRCA mutation carriers is no more than 5% (63). In a more recent casecontrol study from the BCSC, MD was the most prevalent risk factor and had the largest effect on the population attributable risk proportion; the authors estimated that roughly 39% of premenopausal and 26% of

postmenopausal breast cancers could be averted if all women with heterogeneously or extremely dense breasts shifted to scattered fibroglandular breast density (64).

*Risk-conferring components of MD*

Though epidemiological observations suggest a causal relationship between MD and breast cancer risk (65–68), proof of this requires demonstration that a change in breast cancer risk is mediated through the accompanying change in MD (i.e. that MD is an intermediate phenotype). The extent to which reproductive and lifestyle factors influence breast cancer risk through effects on MD is not totally clear (69,70). Rice at al recently found that MD mediated much of the association of early life body size and breast cancer risk, and a significant proportion of the risk associated with benign breast disease and HRT usage (70), but little of the risk associated with a positive family history. It seems paradoxical that MD declines with age whereas breast cancer incidence increases, but cumulative exposure to PMD with increasing age reflects cumulative exposure of breast stroma and epithelium to mitogens and mutagens, whether hormonal, growth factor related or epigenetic (69). This is in accord with Pike's model of breast tissue ageing, in which the cumulative rate of breast tissue ageing describes the age-specific incidence of breast cancer in the US (71) and elsewhere (21,72,73).

The precise nature of the risk-conferring element of MD is unknown. Simplistically, since breast cancers arise from epithelial cells, it is logical to suppose that the greater the MD, the greater the number of epithelial cells and amount of epithelial proliferation. This is consistent with the observation that high MD is associated with increased risk of proliferative lesions and DCIS (74,75), though one study failed to show any statistically significant association between MD and breast cancer risk in women with atypical hyperplasias (76). Some studies, though not all, have shown that age-related atrophy of TDLUs is negatively associated with breast cancer risk and that PMD and failure of TDLU involution are independently associated with breast cancer risk (77–79).

Some groups have found increased stromal expression of ER and epithelial or stromal expression of PR in dense tissue samples from women at population risk or increased risk (80,81). Laboratory studies using mouse models and human tissue xenografts have shown that tamoxifen treatment promotes remodelling of stroma to a tumour-inhibitory phenotype, with reduced extracellular matrix turnover and decreased stromal tissue (82,83).

Non-epithelial components of the stroma and epithelial-mesenchymal interactions appear to be key in the relationship of MD and breast cancer risk. Collagen remodelling and organisation differs in high and low MD breasts and also in different regions of the normal breast (15,84). Boyd et al found that tissue stiffness estimated from mammography was significantly associated with breast cancer and that the addition of stiffness improved the performance of a breast cancer risk prediction model that included percent MD (85). ECM stiffness in known to promote tumorigenesis and collagen alignment adjacent to breast tumours appears to have a direct impact on invasion and metastasis (86). In mouse models, increased stromal collagen and collagen reorganisation is significantly tumorigenic (87), but how exactly aberrant expression of extracellular matrix proteins and increased stromal stiffness promotes tumorigenesis in the dense breast is unknown.

*Does it predispose to a particular sort of cancer?*

A number of studies have evaluated the association of MD with tumour subtypes and oestrogen receptor (ER) positivity or negativity. These studies were highly heterogeneous, but most found no association, others showed stronger associations for ER positive tumours (88–90) and others, stronger associations for ER negative tumours (91). No association with HER2 status was shown in a metaanalysis from 2013 (92).

*The influence of MD on outcomes*

Women with greater MD have a higher risk of dying from breast cancer, largely explained by the increased breast cancer incidence associated with MD (42,91). However, a number of studies have shown that baseline MD may affect outcomes in patients treated for breast cancer. A couple of groups have demonstrated an adverse effect of high MD on local recurrence rates and breast cancer survival, but only in patients who had not received radiotherapy (93,94). Another found that high MD predicted for a greater risk of local recurrence even after radiotherapy, with a HR for high MD breasts compared to low MD breasts of 4.30 (95).

Women with high MD diagnosed with ductal carcinoma in situ (DCIS) appear to be at greater risk of developing subsequent ipsilateral invasive breast cancer (96) and contralateral DCIS or invasive disease (97). Two large cohorts examining the effect of high MD on breast cancer survival did not identify any adverse effects (44,52) whereas a third showed a borderline association (42), but these studies did not specifically address the possible confounding effect of radiotherapy. All these studies provide support for the concept that high MD stroma is able to promote cancer initiation and progression.

**Measurement of MD**

There are two main methods of assessing MD; qualitatively or quantitatively. The former include the Wolfe (36), Tabar (98) and the BI-RADS 5$^{th}$ edition (99) classifications, which assess parenchymal patterns and distributions (figure 1). Quantitative methods include simple visual methods (the BI-RADS 4$^{th}$ edition (1), the Boyd six category classification [SCC] (100) and visual analogue scales (VAS) (101)), semi-automated methods (Cumulus, Madena or planimetry), and fully automated methods. The latter can be area-based or volumetric.

The BI-RADS 4th edition utilised area-based PMD in quartiles (0-24%; 25-49%; 50-74% and 75% areabased density); the SCC is similar but divides the first quartile of PMD into 0%, <10% and 10-<25%. The fully automated area-based methods include AutoDensity, Densitas, ImageJ, iReveal, STRATUS, Libra and MedDensity, but only iReveal has FDA clearance. The best-known volumetric methods, Quantra and Volpara, are model based and have FDA clearance, as does Spectral Density, developed with the Philips Microdose DM system. Other newer fully automated volumetric methods such as $BD_{SXA}$ and CumulusV are used in the research arena. All of the volumetric methods require the raw ('for processing') images from DM units, whereas all the area based methods aside from iReveal function on the processed 'for viewing' images.

Qualitative methods are quick, requiring nothing but the observer's eyes. These pattern based methods appear to add little to visual assessment of area based PMD (102) and suffer from poor reproducibility yet with the BI-RADS 5th edition, a qualitative element of parenchymal pattern classification has been reintroduced to address the possibility of masking. Thus, BI-RADS a denotes almost entirely fatty breasts; BI-RADS b, the presence of scattered areas of fibroglandular density; BI-RADS c, heterogeneously dense breasts where small masses could be obscured, and BI-RADS d, extremely dense breasts, lowering the sensitivity of mammography. A key difference between the BI-RADS 4th and 5th editions is that a breast with under 50% PMD may be classified as BI-RADS c if there is regional MD where small masses could be obscured (figure 3). Tabar described five patterns. Type IV is predominantly dense (equivalent to BI-RADS 3, c or Wolfe P2) and V is uniformly dense, equivalent to Wolfe DY, BI-RADS 4 or d); like the P2 and DY patterns, Tabar types IV and V are associated with increased breast cancer risk.

Simple visual methods of assessing area-based PMD have stood the test of time and numerous studies have confirmed the association with risk, but the SCC and VAS methods are only used in a research setting

(103,104). VAS in particular is useful as, unlike semi-automatic computerised thresholding methods, it is quick, does not require digitisation of mammograms and does not require specific training.

However, as with parenchymal patterns, there are major issues around reproducibility (105). Data from the PROSPR (Population-Based Research Optimizing Screening through Personalized Regimens) consortium indicate that the percentage of mammograms perceived as showing dense breasts (BI-RADS 3 or 4) varies widely across radiologists, from 6.3% to 84.5% (median 38.7%) regardless of patient characteristics. A systematic review of reproducibility of BI-RADS classifications from 2016 found that approximately 1 in 5 women would be categorised differently by the same radiologist at consecutive screens, but more alarmingly this figure was around 1 in 3 when serial mammograms from one woman are read by a different radiologist. Recategorisation from dense to non-dense or vice versa occurred in 13 to 19% of women, a finding which has significant implications in the US (106). Reader experience is important, but even experience cannot overcome the subjectivity of visual assessment, as shown by Lobbes et al (107).

The effect of the change from BI-RADS 4$^{th}$ to 5$^{th}$ editions was analysed by Ekpo et al (108), who found better intra- and interreader agreement with the 5$^{th}$ edition and near perfect agreement when a binary classification (dense or non-dense) was used. The findings of Youk et al were similar but they also showed a significantly greater proportion of breasts classified as dense with the 5$^{th}$ edition (109). Other groups found significantly poorer intra- and inter-reader agreement with the 5$^{th}$ edition (110). A major issue with BI-RADS is that, in most developed countries, most screening age women fall into categories 2 and 3 (or b and c), the interquartile range, where most reader disagreement is found. Since 2 and 3 or b and c differentiate between dense and non-dense breasts, it is difficult to use BI-RADS for clinical decision-making about an appropriate risk adapted screening protocol, though training can improve assignment to BI-RADS categories to a certain extent (111).

*Semi-automated assessment*

The best known method, Cumulus, was developed by Boyd and Yaffe at the University of Toronto in an attempt to overcome the limitations of visual density assessment (112). For many years, Cumulus has been regarded as the gold standard of area-based PMD measurement for research purposes. It is a computer-based semi-automated thresholding technique requiring digitisation of SFM, a major drawback. The user first defines the chest wall, masking the pectoral muscle, and segments the entire breast area. An interactive thresholding tool is then used to define the breast parenchyma; the output is PMD (figure 4). With training, the reproducibility of Cumulus is excellent within and between readers and there is generally good agreement both between right and left breasts and MLO and CC views (113,114).

Cumulus PMD has been repeatedly validated and shown to correlate with breast cancer risk in numerous studies (58,113,115). However, as a clinical tool it is impractical. The same is true of Madena, which also requires human input for segmentation into dense and non-dense tissue (113). Later iterations, AltoCumulus and CirroCumulus, work on DM images and one area of active research is into the effect of altering thresholding in DM images, with higher thresholds appearing more predictive of risk (116).

*Automated volumetric methods*

Radiographic factors and positioning can have a profound effect on measured PMD (figure 6) and concerns about the basic relationship between area-based PMD from a 2D mammographic projection and breast cancer risk persist, as there is no information on breast thickness and therefore volume of dense tissue (figure 5), which might be expected to be a more relevant metric (117). Thus there was a pressing need for fully automated volumetric methods of measuring MD and the advent of DM has made this possible. The best known of these methods are Quantra and Volpara, a multi-vendor method.

Quantra was the first commercial method, based on research by Highnam and Brady, who continued work on their algorithm to develop Volpara a few years later. Both are model based, but work in slightly

different ways to calculate dense volume, total breast volume and percent volumetric MD (VBD) from individual pixel intensities and known xray attenuations. Quantra uses an absolute model and includes the skin; Volpara uses a relative physics model by finding a pixel of pure fat attenuation as an internal reference (118). As well as yielding dense and total breast volumes, Volpara gives a density grade (VDG) that can be aligned to the 4$^{th}$ or 5$^{th}$ edition of BI-RADS and Quantra gives a similar measure (figure 7). Both methods have been shown to correlate well with breast cancer risk, particularly Volpara (115,119).

$BD_{SXA}$ uses a phantom step wedge that requires placement on the image receptor and which is compressed to the same degree as the breast so as to provide grey scale references for each pixel on the mammographic image for calculation of volumetric MD (120); CumulusV calculates volumetric MD from compressed breast thickness and xray attenuation after calibration of the DM unit using breast – equivalent phantoms (121). Both are used for research purposes.

The evidence suggests that automated volumetric assignment to a density grade is very consistent (122,123). In one study of trained readers, the number of mammograms classified as dense (BI-RADS 3 or 4) ranged between 25 to 50% depending on the reader, whereas a cut-off of 22% volumetric density with Quantra correctly predicted 89% of non-dense and 90% of dense breasts (123). There are occasions where fully automated methods may not work (for example in very large breasted women or women with implants); Volpara can also produce erroneous readings with very high MD where it is not possible to identify a pure fat-containing pixel. Alternative approaches have been explored by the Nijmegen group to improve density estimation in this situation (124). Other technical issues that can profoundly affect measurements include paddle tilt, resulting in variation in compressed breast thickness and therefore computation of VBD; in this situation, correction for tilt is essential (125).

The consistency of MD measurement in serial mammograms is also clinically relevant. In a study of women from the Dutch screening programme, MD of serial mammograms was assessed visually and with Volpara

(126). Better agreement was found with the latter, with fewer instances of implausible changes from non-dense to dense categories (2.8% versus 4.2% of cases) and a significantly higher interexam agreement for VBD compared to group visual reading. This is important in considering which method might be appropriate to inform screening protocols. VBD has been used to quantify the potential risk of masking as well; in a retrospective study of screen detected and interval cancers both VDG and VBD had a stronger linear relationship with mammographic sensitivity than BI-RADS classification (127) and both automated measures yielded higher odds ratios for interval cancers than BIRADS, suggesting that they could be used to stratify women into a density-adapted protocol.

**Other measures of breast density and composition**

*Fully automated area-based methods*

A number of fully automated area-based methods of measuring PMD have been developed, some of which are commercially available and others of which are freely downloadable. These include an ImageJ based method (developed at the Karolinska), AutoDensity (University of Melbourne), LIBRA (using fuzzy c-means segmentation and developed at the University of Pennsylvania), STRATUS (based on machine learning, also from the Karolinska) and MedDensity, developed at the University of Genova. These can work with SFM, DM and digital breast tomosynthesis (DBT).

iReveal and Densitas (128) are commercially available (figure 8). The main advantage of these systems is that they utilise processed ('for viewing') images without the necessity for storage of raw images, a major constraint on PACS storage systems. However, there is a paucity of independent research using these methods, though one case-control study using Densitas found better risk prediction than a clinical risk model (128). The image type (raw, analogue-like or processed) may also affect output and association with breast cancer risk (129). Findings from a number of other studies also support the notion that the

same VBD tool should be used in serial measurements or when different populations are compared; Eng et al found that VBD readings were markedly different between Volpara and Quantra (figure 9) (115), the latter yielding much higher within-woman measurements, a finding replicated by Morrish et al (130).

*DBT and synthetic 2D DM*

With increasing use of DBT either as an adjunctive or primary screening method, it is important to be able to assess MD as reliably as with conventional 2D DM. Though BI-RADS can be used, it is challenging to evaluate a series of reconstructions (131). One group has developed a fully automated software using a thresholding method to analyse MD of each DBT reconstructed slice (132). This demonstrated lower PMD for DBT than DM. Subsequently this software, MedDensity, was used to compare PMD readings in a cohort of women with both DM and DBT studies (133). The difference in PMD between DM and DBT differed across BI-RADS categories in a non-linear fashion, with least difference in BI-RADS 3 breasts (3.5%) and most in BI-RADS 1 and 4 breasts (16 and 18% respectively). The group also compared PMD in a cohort of women who underwent DM, DBT and breast MRI (134). Though there was a strong positive correlation between all three modalities, differences in PMD between DM and DBT and between DM and MRI were highly significant, with DM yielding 15% higher PMD, whereas no significant differences were found between DBT and MRI.

Other groups have used the central projection of the DBT acquisition to estimate PMD, showing good correlation and substantial agreement with PMD measured from DM using Cumulus (135).

Volpara version 1.5.1 is able to derive volumetric MD from the central projection and compressed breast thickness. A feasibility study using paired DM and DBT images obtained during one compression (136) demonstrated near perfect agreement for calculated breast volume, but small though statistically significant differences for dense volume and hence VBD. Other groups have used a similar modelling

approach to derive VBD using each of the DBT projections, again with closer agreement between VBD derived from MRI and DBT than between DM and either DBT or MRI (137).

BI-RADS density classification of synthetic 2D mammograms derived from DBT has been compared with with standard DM, with good agreement for individual readers (80%; Cohens kappa 0.73) and excellent agreement when images were dichotomised into dense and non-dense (92%) (138). Good agreement has also been demonstrated for Hologic Selenia Dimensions 'C-View' synthetic mammograms and standard Selenia DM using LIBRA (139).

*Spectral mammography*

Since fibroglandular and adipose tissues have different effective atomic numbers (Z), spectral decomposition can be used to quantify the thickness measurements of each tissue type, without the need for assumptions about breast thickness, height of the compression paddle or the necessity to identify a purely fatty pixel. This can be done using dual energy techniques, which have been shown to yield accurate estimations of breast density (140), though at the cost of slightly higher radiation doses. It is also possible to analyse breast composition, with accurate experimental separation of water, protein and lipid components (141). Spectral mammography with energy-resolved photon counting detectors eliminates the need for two exposures, reducing radiation dose. A recent comparative study using BIRADS, Cumulus, a fuzzy C-means segmentation and spectral mammography material decomposition showed excellent right-left correlation for the latter, with much higher precision than for the other techniques (142), an observation replicated in a recent study from Sweden (143).

*MRI*

Breast MRI is an attractive means of measuring breast composition; it is a 3D technique, with no tissue overlap and no necessity for compression or irradiation and has been regarded as the ground truth for

measuring the accuracy of volumetric mammographic techniques (144). With Dixon techniques it is possible to assess breast composition with measurement of fat and water volumes (145). In a study of women under 30 and their mothers, Boyd found that PMD was strongly correlated with percent water at MRI and that percent water in the young women was strongly positively correlated with height and maternal percent water, suggesting that MD in middle age may well reflect influences on growth and development earlier in life (146).

The MRI sequence used can have a profound effect on measured % FGV (147,148), and bias field correction is essential since without it, non-uniformity of the B1 field and resultant variations in signal intensities across the breast makes accurate segmentation difficult (figure 10). Furthermore measurement of % FGV necessitates accurate and consistent segmentation of the whole breast volume, which is surprisingly difficult. Landmarks are often not specified and where they are, various boundaries have been used including a ventral line drawn coronally from the anterior border of the pectoral muscle or a line following the curve of the pectoral muscle (figure 10). Semiautomated methods require operator intervention, which is time consuming and subjective (149,150). Model-based methods using templates or atlas-based algorithms are preferable (151,152) and could efficiently and reliably process large datasets for FGV estimation. Recent work from the UCL group suggests that the optimal automatic segmentation is highly subject-specific and one size may not fit all (153).

Small studies looking at short term reproducibility after repositioning have shown excellent agreement (148,154). As expected there is strong correlation with MD measures, but the level of agreement varies depending on whether area-based or volumetric measures of MD are used (134,154,155).

*Ultrasound (US) and US tomography*

US reliably differentiates adipose and non-adipose tissues, is non-invasive, non-ionising and readily available and therefore could be useful in assessment of breast composition. In a reader study of 40

patients with 328 reads, 2D US was used to assess the relative proportions of adipose and glandular parenchyma in each quadrant of the breast. There was good correlation with BI-RADS MD and exact agreement of 86% when scans were dichotomised into dense or non-dense (156).

3D US has also been evaluated. Moon et al found that percent density and breast volume derived from ABUS correlated very well with those derived from MRI, though the latter yielded substantially larger volumes (157). However, the deformation of the supine breast and possibility of overlapping volumes makes this technique potentially inaccurate and poorly reproducible. The same group has developed a rapid volume density analysis (158) in which there was good correlation with whole ABUS methods. An alternative means of extracting dense volumes from ABUS uses the rib shadows to define the breast volume and shows potential (159).

A more promising technique uses ultrasound tomography (UST) to calculate parameters such as the volume-averaged speed of sound, VASS, which is density-dependent; the higher the density, the higher the in-vivo US speed. A series of US tomograms is collected with the patient lying prone with the breast pendent in a water bath. Initial results demonstrated a strong positive association between VASS and BIRADS MD, confirmed in larger studies comparing VASS with thresholding techniques (160,161). VASS was positively correlated with Cumulus dense area and reduced with age and postmenopausal status, in line with the corresponding reduction in PMD. A subsequent operator study showed excellent inter-and intra-rater reproducibility (162) and a larger study of women with negative mammographic screens demonstrated that sound speed measures showed consistent associations with PMD (163). More recently a comparative study of healthy volunteers showed an extremely strong correlation between VASS and percent water density at MRI using a Dixon technique (figure 11) (164).

*Optical imaging based techniques*

Though only being investigated in the research arena, optical imaging techniques offer the possibility of tissue characterisation. Light in the visible and near-infrared part of the spectrum is absorbed and scattered differentially by fatty and dense breast tissues, which contain chromophores in the form of water, lipids, deoxyhaemoglobin and oxyhaemoglobin. More scattering, water associated absorption, higher total haemoglobin and deoxyhaemoglobin is seen in dense breast tissue. Various forms of optical spectroscopic techniques have been used, of which the best known is diffuse optical spectroscopic imaging (DOSI). Using this technique promising correlations with MD, factors associated with high MD and MRI FGV have been identified by some research groups (165–167).

*Texture*

While MD measures give a global overview of breast composition, evidence is emerging that parenchymal patterns are indicative of breast cancer risk and performance of DM at screening (168). Preliminary analysis from the ACRIN PA 4006 trial found that parenchymal complexity at DM was positively associated with false positive recall rate; no such association was found with DBT. Early work suggested that parenchymal patterns were associated with breast cancer risk and that this effect was independent of MD (45,169,170). However, many of these early studies suffered from poor reproducibility compared to quantitative estimation (171).

Texture in a medical image can be defined as a computerised mathematical method of describing spatial variations in pixel intensity, which may not be appreciable with the naked eye. Texture analysis (TA) is automated and thus subjectivity is not an issue. Within the last decade there has been renewed interest in texture analysis (TA) of mammograms and its role in risk assessment, with many novel computerised approaches, reviewed in more detail by Gastounioti et al (172).

In most early studies of TA in mammography a single region of interest (ROI) was utilised in the retroareoalar area but recently attempts have been made to capture texture features across the entire breast using multiple ROIs or a lattice structure, which may improve risk assessment. A detailed description of the texture descriptors used is outwith the scope of this article, but most studies have used grey level histogram features (first order statistics), denoting the distribution of pixel intensities, grey level co-occurrence matrices (which consider spatial relationships of signal intensities), run length measures (which assess uniformity by measuring the number of pixels with the same signal intensity in specified directions), structural measures (characterising tissue complexity) or spectral features, the latter using spatial frequency transforms to characterise repetitive texture structures. These techniques were initially developed for use on digitised SFM, but have now been applied to DM and a number of fully automated methods have been developed using a cross-validation approach.

Early prospective studies on SFM showed moderate relative risks with texture measures and no additional improvement in discrimination with subsequent addition of MD measures, confirmed in many retrospective studies using increasingly complex texture descriptors and combinations of TA features (173,174). Nielsen et al have developed a mammographic texture resemblance marker (MTR) which demonstrated good discrimination in two completely different cohorts, suggesting the measure is generalisable; the best discrimination was achieved with an aggregate of MTR and Cumulus PMD, with an area under the ROC curve (AUC) of 0.66 (175).

Studies using DM have attained AUCs of between 0.73 (176) to 0.85 (177), the latter using a complex combination of texture features. Some TA features may be more predictive of certain tumour subtypes (ER positivity or negativity) (178) and whereas MD does not appear to be predictive of risk in women with BRCA mutations, TA features may be predictive for mutation status (179,180).

Though multiparametric TA appears highly predictive of risk, comparative studies on one large dataset are lacking and more research is needed into optimised methodology, including location and size of ROIs chosen. It is also important to know whether the image format (raw or processed) and vendor unit affects the predictive abilities of TA, as this would have a significant impact upon the design of prospective multicentre and multivendor studies (181,182). Nonetheless the results are encouraging, particularly the fact that TA appears to confer information on risk separate from that provided by MD (175,180). Future research is likely to focus on the relative contributions of density, texture and parenchymal patterns, a recent case-control study finding that while BI-RADS density, Tabar classification and texture scores were all predictive, the AUC was greatest for a combination of the three
(183). Deep learning is also likely to prove highly valuable in the application of TA to raw DM images (184) and TA lends itself to radiogenomics. Finally, with increasing use of DBT in the screening setting, a major challenge is application of TA to volumetric DBT data. Preliminary data applying various TA measures to an ROI from the retroareolar region has shown that texture features are strongly correlated with MD at DBT, but a relationship with risk has yet to be proven (185,186).

**Clinical implications of MD**

*Risk adapted screening protocols*

Public recognition of the impact of MD on mammographic screening performance, together with concerns over overdiagnosis, has resulted in a significant shift in the perception of the utility of mammographic screening and a demand for density-tailored screening. Possible strategies to deal with this include increased frequency of mammographic screening; alternative tests (either DBT or abbreviated MRI techniques or, to a lesser extent, molecular breast imaging) or supplemental tests such as whole breast ultrasound (either automated or hand-held). Space precludes a detailed review here (please see reference (106)) but some key considerations are summarised below.

In the absence of definitive results from randomised controlled trials of screening protocols according to risk of masking, simulation studies can provide useful information on risk-benefit analysis. Schousboe et al considered costs per quality-adjusted-life-year (QALY) of various screening strategies according to age, breast density, family history or a previous breast biopsy. They found that annual mammography was not cost-effective for any age group regardless of age or breast density; biennial mammography cost under $50,000 per QALY for women aged 40-49 years with BI-RADS 3 or 4 density and a previous breast biopsy or family history (187). Another study found that for women at 2-4 fold increased risk (very dense breasts and/or a family history) annual screening from the age of 40 had comparable risks and benefits to those of average risk women undergoing biennial screening from the ages of 50 and 74 years (188). A further study estimated screening outcomes for women aged between 50 to 74 years for various mammographic screening intervals by breast density and risk (189). Whereas screening benefits and overdiagnosis rates increased with breast density, false positives decreased. For women at average risk and low breast density, triennial screening was as effective as biennial screening. Conversely, for high-risk women and BI-RADS 3 or 4 density breasts, annual screening averted more deaths, but harms were two-fold higher.

DBT is now being evaluated as a stand-alone technique for screening in women with dense breasts. The development of synthetic 2D images of comparable quality to standard DM (except for microcalcifications) goes some way to relieve concerns about radiation dosages (190,191) and DBT with either synthetic or standard 2D DM is substantially more sensitive than 2D DM alone (192). Subgroup analysis of the UK TOMMY trial demonstrated that DBT was significantly more sensitive than 2D DM in women with more than 50% MD (93% and 86% respectively, p 0.03) (193). A rapid review and metaanalysis of DBT performance in women with dense breasts yielded a pooled incremental cancer detection rate (ICDR) for DBT over 2D DM of 3.9/1000 screens in four prospective studies set in population-based European screening programmes (194). However, there is to date insufficient evidence

on the biological importance of additional cancers detected by DBT, with most being grade 1 or 2 spiculated cancers. Thus, DBT could add to the problem of overdiagnosis and as yet the impact of DBT on T/N stage and interval cancer rates, a proxy for mortality reduction, is unknown. Encouragingly, initial results from the University of Pennsylvania suggest that the annual cancer detection rate with DBT is maintained, with significant recall reduction, a higher PPV for recall year on year and, and a suggestive decline in interval cancer rates (195).

The available evidence to date suggests that supplemental US detects more cancers than DBT (106), with an ICDR of approximately 4/1000 US screens, but whereas studies of DBT included women with no other risk factor than dense breasts, most studies of supplemental US have been in women with additional risk factors as well (196–198). A key concern is the false positive rate and low PPV; in the ACRIN 6666 study, the positive predictive value for biopsy (PPV3) was 16% compared to 38% for mammography alone (197). The number of screens needed to detect one cancer was 127 for DM, 234 for supplemental US but only 68 for MRI after negative DM and US. Though the risk of false positives decreased between prevalent and incident US screens it was still substantial, with only 7.4% of those biopsied after incidence screening US proving to have cancer. The cancer detection rate year on year was maintained, but doubts have been raised about the cost-effectiveness of screening US for women with dense breasts (199).

Results from the Japanese J-START study suggest that supplemental US may decrease interval cancer rates (200), though results by breast density were not given. The Italian prospective ASTOUND trial comparing DBT and US for adjunctive screening in women with dense breasts recently reported interim results, with a cancer detection rate of 7.1/1000 screens for US and 4/1000 for DBT (201). In this study, false positive recall and biopsy rates for US and DBT were comparably low, reflecting the fact that many of the US screens were incident screens with availability of prior studies. Potential advantages of US over DBT include wide availability, good tolerance and lack of ionising radiation; on the other hand, even with an

automated system there is still operator dependency, there is often a need for supplemental hand-held US, interpretation times are long and there is a lack of personnel to read the scans (202).

In her systematic review of supplemental screening in women with dense breasts, Melnikow found that breast MRI had high sensitivity, variable specificity (up to 94%) and PPV (3-33%) (106). There is little doubt that MRI will detect additional cancers, as shown by Berg et al (197), where the supplemental yield of MRI after negative DM and US was 14.7 cancers/1000 screens, but the prohibitive cost of MRI together with resource constraints mean that it is not cost-effective to offer screening MRI for any group other than the high risk group (lifetime risk over 25-30%). This may change with the advent of abbreviated MRI protocols (203,204). The ongoing Dutch randomised controlled trial of supplemental MRI screening in women with dense breasts, DENSE, aims to assess reductions in interval cancer rates as well as the number of MR screen-detected cancers (205). An important consideration will be false positive recall rates, since many lesions may only be visible at MRI, necessitating either short-term follow-up or MR guided biopsy, neither of which are desirable in a cost-limited healthcare system. Another critical factor is client acceptability; in the ACRIN 6666 study, 42% of the women offered supplemental screening MRI declined it, even though possible costs to the women were covered by the study (206). Reasons cited included claustrophobia and time constraints, both of which might be partly alleviated by abbreviated MRI protocols. Studies addressing the impact of such protocols have shown dramatic reductions in MRI room usage (207).

Like MRI, contrast-enhanced spectral mammography (CESM) could detect more biologically important vascular cancers (208). It appears to be as sensitive as MRI but quicker, cheaper and better tolerated, despite the fact that it involves irradiation and an injection of contrast material (209,210).

Currently most international guidelines do not recommend supplemental screening for women with dense breasts and no other risk factor, though the American College of Radiology considers that supplemental

US is appropriate in women with dense breasts and one other risk factor (211). This is supported by the findings of a recent study evaluating the effect of breast density and Breast Cancer Surveillance Consortium (BCSC) 5-year risk on interval cancer rates (212). In this prospective study, women with heterogeneously or extremely dense breasts but low to average BCSC 5 year risk had acceptably low interval cancer rates. Thus global MD alone may be insufficient reason to offer supplemental screening; increasingly the focus is on regional MD and breast tissue organisation (213) and volumetric density maps may be used to define women at increased risk of an interval cancer (214). It has been shown that cancers are far more likely to arise in focal areas of MD identified on prediagnostic screening mammograms (215).

*MD as a measure of risk*

While MD is a very strong population-attributable risk factor, its value in determining whether an individual woman will develop breast cancer is limited. The ability of any risk assessment tool to predict whether an individual will develop breast cancer is judged by the ROC curve; the greater the area under the curve, or *c*-statistic, the more discriminatory a risk factor is. The best known clinical tools are the Gail, Claus, BRCA-Pro, Boadicea and Tyrer-Cuzick models (216) and in the literature these have *c*statistics between roughly 0.6 and 0.7, depending on the population evaluated and how well calibrated the tool is for that population. Until very recently, MD was not included in any of the commonly used risk prediction models. Early studies of the addition of MD used either BI-RADS or planimetric classifications of MD and the Gail model and consistently demonstrated a small increase in the *c*- statistic from around 0.6 to 0.65 (217–220). Tice et al, in a study of over 1 million ethnically diverse women found that addition of BI-RADS density to the Gail model improved the *c*-statistic from 0.61 to 0.66, but importantly, with low MD the 5-year risk was under 1.67% unless there was another risk factor such as family history or age >65 years (219). This suggests that MD could help inform screening programmes for individual women by defining a low-risk group of women for whom little or no screening is needed. The addition of percent MD (assessed

by VAS or 5% bins) adjusted for age and BMI (the density residual) to the Tyrer-Cuzick and Gail models improved the AUC in two recent studies, both of which also demonstrated that MD as a univariate risk factor was slightly more discriminatory than the Tyrer-Cuzick model alone (221,222). Other studies have used area based thresholding techniques (ImageJ) with similar results (223).

A measure of MD has now been added to the Breast Cancer Surveillance Consortium risk model (version 2), which uses BI-RADS, and to the Tyrer-Cuzick model, version 8, which allows use of BI-RADS, VAS or an automated density tool such as Volpara (224). It might be anticipated that automated volumetric measures of MD could improve risk assessment for individual women, especially as many women fall into the middle two BI-RADS categories, but available data from the PROCAS study suggests that Volpara VBD performs no better than VAS (103,224). However, visual measures may not be reproducible with different readers. Volumetric and area-based methods, though correlated, are not the same and might not be equivalent when used in a model; indeed, other studies comparing area-based and volumetric methods have shown stronger associations with volumetric measures (115,225,226).

One recent study found that the combination of Volpara dense volume and BI-RADS category refined the BCSC risk model more than either measure alone; among women with BIRADS 4 breasts, only those with 3$^{rd}$ and 4$^{th}$ quartile dense volume were at significantly increased risk. Adding Volpara dense volume to the BCSC v.2 risk tool (which includes BI-RADS density) improved the *c*-statistic from 0.614 to 0.639 ($p<0.001$) and women with BIRADS d category breasts and first quartile Volpara dense volume had a 5 year risk under 1.8% (227). This important research suggests that automated volumetric measures could be used to better identify women at risk and refine screening strategies for such women.

However, it is not clear which metric is most informative in risk prediction; some studies suggest that PMD or VBD are more discriminatory than absolute measures of MD and it is unclear whether the nondense area is important. Furthermore, though studies have clearly shown that volumetric measures are

associated with risk, the strength of the association varies considerably (115,119,225) with some studies demonstrating highest ORs with volumetric measures and others, much higher odds ratios with BI-RADS assessment. The BIRADS classification yields a maximum OR of around 4 in most studies, which is not dissimilar to Cumulus, whereas the maximum OR with the volumetric methods (comparing the lowest quintile of MD with the highest) is 8 (115). Additionally, there are striking differences between volumetric methods, even though they are well correlated (115,228). It should also be borne in mind that the widely cited relative risks of 4 to 6 refer to women at the extremes of MD; the highest categories of MD confer a relative risk of nearer 2-fold when average MD is considered.

*MD as a biomarker for clinical interventions*

Tamoxifen, a selective ER modulator (SERM), was shown in the IBIS-1 study to reduce breast cancer risk in women at increased risk (229). Women on tamoxifen whose mammograms demonstrated a reduction in MD of more than 10% had a 63% reduction in breast cancer risk; no such reduction occurred if the fall in MD was less than 10% (6). This protective effect has been sustained at follow-up (230). Similar findings were reported by Li et al (231) in a study of postmenopausal women receiving adjuvant tamoxifen for 10 years; patients on tamoxifen with a 20% reduction in MD had a 50% reduction in risk of breast cancer death compared to women with stable MD, as measured by a Cumulus-based thresholding method. Nyante et al, in another case-control study, also showed that reductions in breast cancer death on tamoxifen only occurred in patients in the middle and upper tertiles of MD at baseline prior to commencement of adjuvant therapy (232). Similarly reduction in MD in premenopausal women on tamoxifen is associated with a lower risk of locoregional or distant recurrence (233).

It has also been shown that in patients receiving adjuvant tamoxifen, a reduction in MD of more than 10% as measured with Cumulus decreases the odds of metachronous contralateral breast cancer at follow-up by 55%; no such protective effect occurred in women with smaller reductions in MD (234). This suggests

that MD could perhaps be used as a biomarker for the protective effect of tamoxifen, either as adjuvant therapy or as chemoprevention for high-risk women. At least one small study has correlated the likelihood of breast cancer recurrence after adjuvant tamoxifen with reductions in MRI FGV, finding that PMD reduction on tamoxifen was the only independent factor associated with recurrence; mean reduction was around 20% in the group without recurrence and only 2-6% in the group with recurrence (235).

The Biomarker Definition Working Group of the National Institute of Health in the US defines a biomarker as any objectively measurable characteristic that could either indicate an underlying physiological or pathological process, such as breast cancer risk (in which case it is regarded as prognostic), or evaluate the response to an intervention (eg. tamoxifen as chemopreventive or adjuvant therapy), where it is predictive. MD appears to meet many of the criteria for a biomarker, since it is quantifiable, serially measurable and applicable to preventive and adjuvant scenarios. Thus it is critical to be able to measure MD accurately and reliably, such that the size of any effect secondary to the intervention easily exceeds any measurement error. Results of studies using fully automated volumetric measures of MD are few, but Engmann et al have shown that in women with breast cancer treated with adjuvant tamoxifen (premenopausal) or an aromatase inhibitor (postmenopausal) there were significantly greater reductions in dense volume and VBD in cases compared to controls using Volpara and Quantra; reductions were greatest when baseline VBD was more than 10% (236). Concerning other SERMs such as raloxifene, and AIs, the data for the use of MD as a biomarker is less robust and reported reductions in MD appear to be less and very heterogeneous. However as AIs are generally given in the postmenopausal setting, it is not surprising that effects on MD are less profound, and greatest reductions in MD on tamoxifen are seen in those with greatest baseline MD. Many questions remain unanswered; such as what threshold reduction in MD predicts a favourable outcome; which method should be used (is BI-RADS or VAS sufficient?); and

if automated methods are used, which parameter is most discriminatory (absolute or percent dense areas/volumes)?

**Unanswered questions**

Though MD undoubtedly shows promise as a biomarker many issues need to be addressed before MD can be used either to determine screening strategy or the value of risk reducing interventions.

A critical question is the consistency of measures of MD and measurement error; if for example the reduction in VBD associated with response to tamoxifen was 6% but similar variability could result from changes in positioning, compression or use of a different mammographic unit, measured reductions would be meaningless. A number of studies have investigated this question. With BI-RADS and PMD estimation, variability within and between observers after short term reimaging is substantial especially in women with higher MD (237). Volumetric measurements do appear more reproducible in the short term, especially with Volpara and Quantra compared to Cumulus and CumulusV, but these studies are small (238,239).

Another key question is whether the different manufacturer algorithms affect visual or volumetric measures of MD. The automated methods rely on image pixel intensity values, so alterations in relative values could affect PMD measurements whether area-based or volumetric. Two studies have found that visual assessment of PMD may be higher on GE than Hologic mammograms taken one year apart in different cohorts of 100 and 40 women respectively (240,241), to the extent that BI-RADS categorization was different in 10% of the women in the larger study; however, there were no significant differences in VBD or VDG in either study.

A further consideration is the effect of the normal physiological reduction in MD over time in a given woman (21,77). Though MD changes track, women with higher MD undergo greater changes at the time of the menopause and it is baseline MD that is most strongly predictive of risk (61,242). However, it

remains unclear whether absolute or percent measures of MD should be measured. In a meta-analysis of 13 case-control studies, percent dense area was more predictive of risk than absolute dense area in premenopausal and postmenopausal women, and though absolute nondense area was inversely associated with risk, it was not clear whether this effect was independent of absolute dense area (243). Lokate et al on the other hand found that in postmenopausal women, non-dense area was independently associated with breast cancer risk; the highest risk was found in women with dense and non-dense areas greater than the median (244). In this study, MLO views were used and it is possible that the measured fat included non-breast axillary fat – which would be increased with high BMI, a risk factor for postmenopausal breast cancer. In addition more work is needed to establish whether MD is solely a risk factor for ER positive cancers; Yaghjyan et al found that absolute dense area was associated more with non-basal tumours and non-dense area had stronger negative associations for ER negative and basal tumours (245). In a case-only study from Sweden, women designated high risk by the TyrerCuzick model and a SNP polygenic risk score were more likely to be diagnosed with good prognosis tumours (ER positive, low grade) especially under 50 years, whereas area based MD was not associated with any of the measured tumour-related prognostic factors (246). Using volumetric measures, absolute measures of density were found to be more predictive than percent measures, but the best results were obtained with a combined area/volumetric model (247). A recent publication also found that women with HER2 positive tumours had the highest volumetric PD and this association was also significant with quantitative measures (248); more research along these lines is needed.

**Conclusions**

MD has profound implications for all those involved in the care and management of women with, or at risk of, breast cancer. It is key in the evolution of risk-adapted personalised screening, density-tailored imaging and evaluation of the efficacy of therapeutic interventions. With ever more robust automated

measures of MD and parenchymal texture in the pipeline, and the development of non-mammographic methods of assessing breast composition, the future for this field of breast imaging is dynamic and full of promise.

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Figure legends

1. Figure 1. Examples of differing mammographic density.

   a) BI-RADS 4$^{th}$ edition 1; 5$^{th}$ edition, a. Wolfe N1, Tabar II.

   b) BI-RADS 4$^{th}$ edition 2; 5$^{th}$ edition, b. Wolfe P1, Tabar I, III.

Figure 1{ PAGE * MERGEFORMAT }

c) BI-RADS 4th edition, 3; 5th edition, c. Wolfe P2, Tabar IV.

d) BI-RADs 4th edition, 4; 5th edition, d. Wolfe DY, Tabar V.

2. Figure 2. Effect of HRT. Bilateral MLO views a) before and b) 1 year after commencement of HRT.

3. Figure 3. Effect of change from BI-RADS 4th edition to 5th edition. Left MLO and cc views from two different patients, both classified as BI-RADS c; in the fourth edition the first patient would be BI-RADS 3 and the second, BI-RADS 2. 3a, b: BIRADS 3 and c. 3c,d: BI-RADS 2 and c.

4. Figure 4. Screen capture of user graphical user interface for Cumulus. Red lines denoted masked chest wall and skin; interactive thresholding tool enables segmentation of MD. (*Image reproduced courtesy of Cumulus*)

5. Figure 5. a) Schema demonstrating difficulty in assessing volumetric MD from a 2D projectional image. i. The second MLO has double the amount of MD ii. On the cc view, area-based percent MD appears the same for both MLOs. b) Right MLO, BI-RADS categorisation 3 or c. c) On the corresponding axial T2 weighted MRI, there is well under 50% area based percent MD.

6. Figure 6. Effect of positioning. Same patient, right cc views I year apart. Positioning is better in a) than b) with more of the breast pulled on. Percent MD will be lower in b). The human eye can appreciate the difference in positioning whereas an automated or semiautomated programme may not.

7. Figure 7. The output from Volpara version 1.5. (*Image reproduced courtesy of VolparaSolutions*).

8. Figure 8. The outputs from Densitas. (*Image reproduced courtesy of Densitas*).

9. Figure 9. Taken from Eng et al. Distribution of MD by area-based and volumetric methods from control subjects. Notice highly significant variation in area-based and volumetric percent MD between Volpara and Quantra in particular. (*Image reproduced with permission of BioMed Central*)

10. Figure 10. a) Note effect that different methods of segmentation of the chest wall could have on breast volume and therefore percent fibroglandular volume. b) $B_1$ inhomogeneity results in shading in the posterior right breast and flare in the medial left breast in particular. Acceptable segmentation with user-defined thresholding will not be possible without bias field correction.

11. Figure 11. a) The 'SoftVue' unit for ultrasound tomography (UST) b) coronal reconstructed image, pixel intensity denoting speed of sound. Note ready differentiation of fibroglandular parenchyma from adipose tissue.

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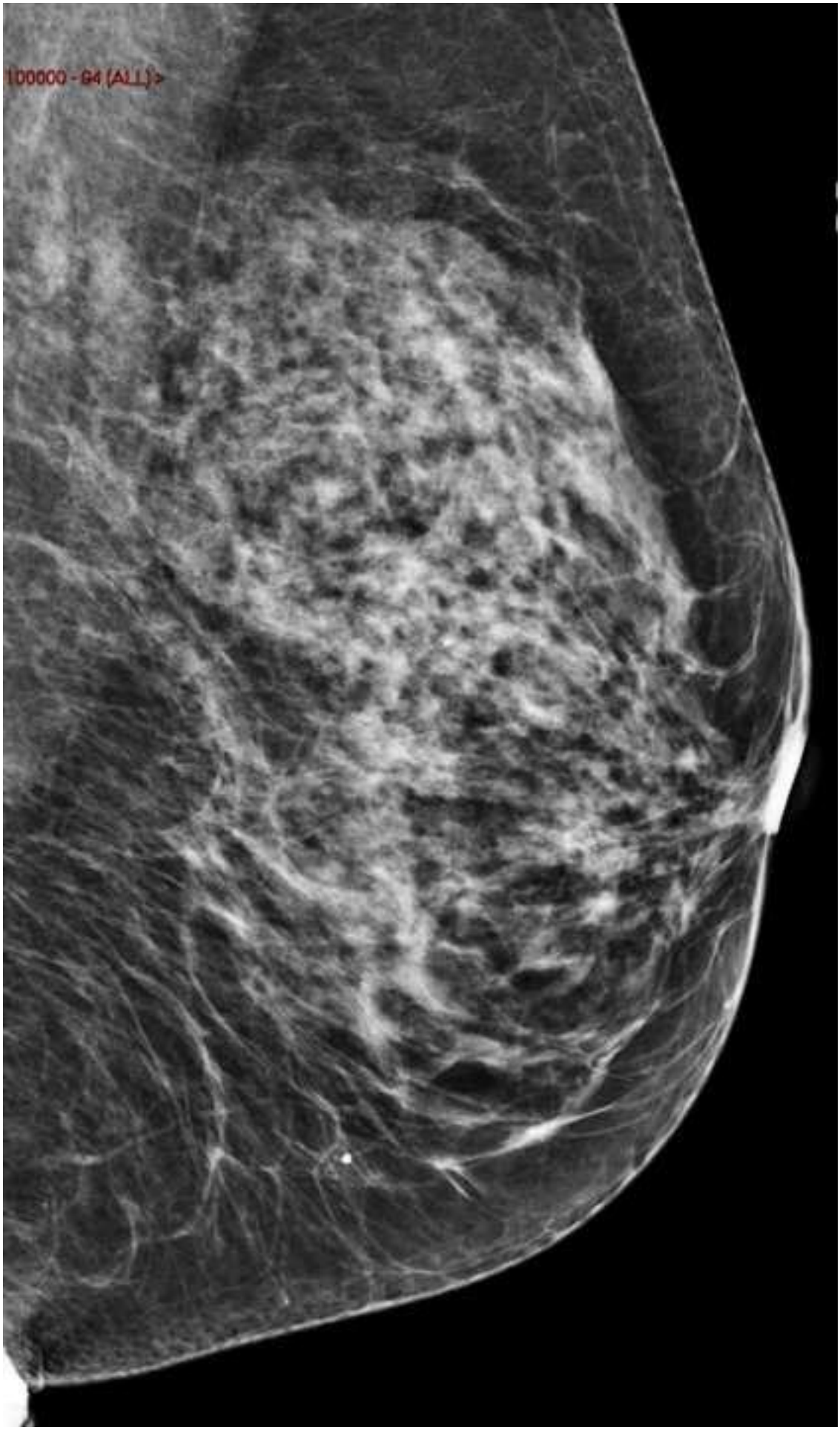

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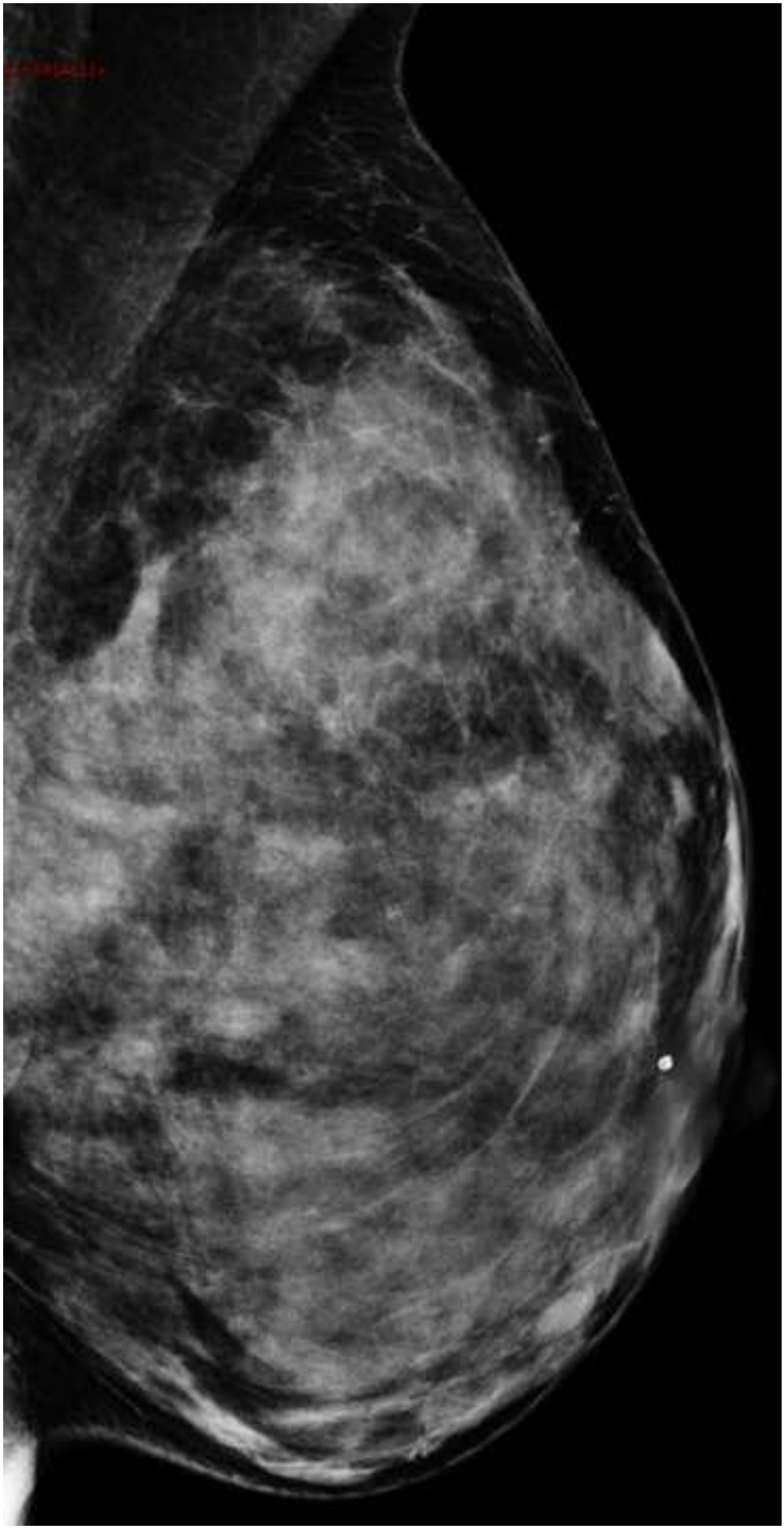

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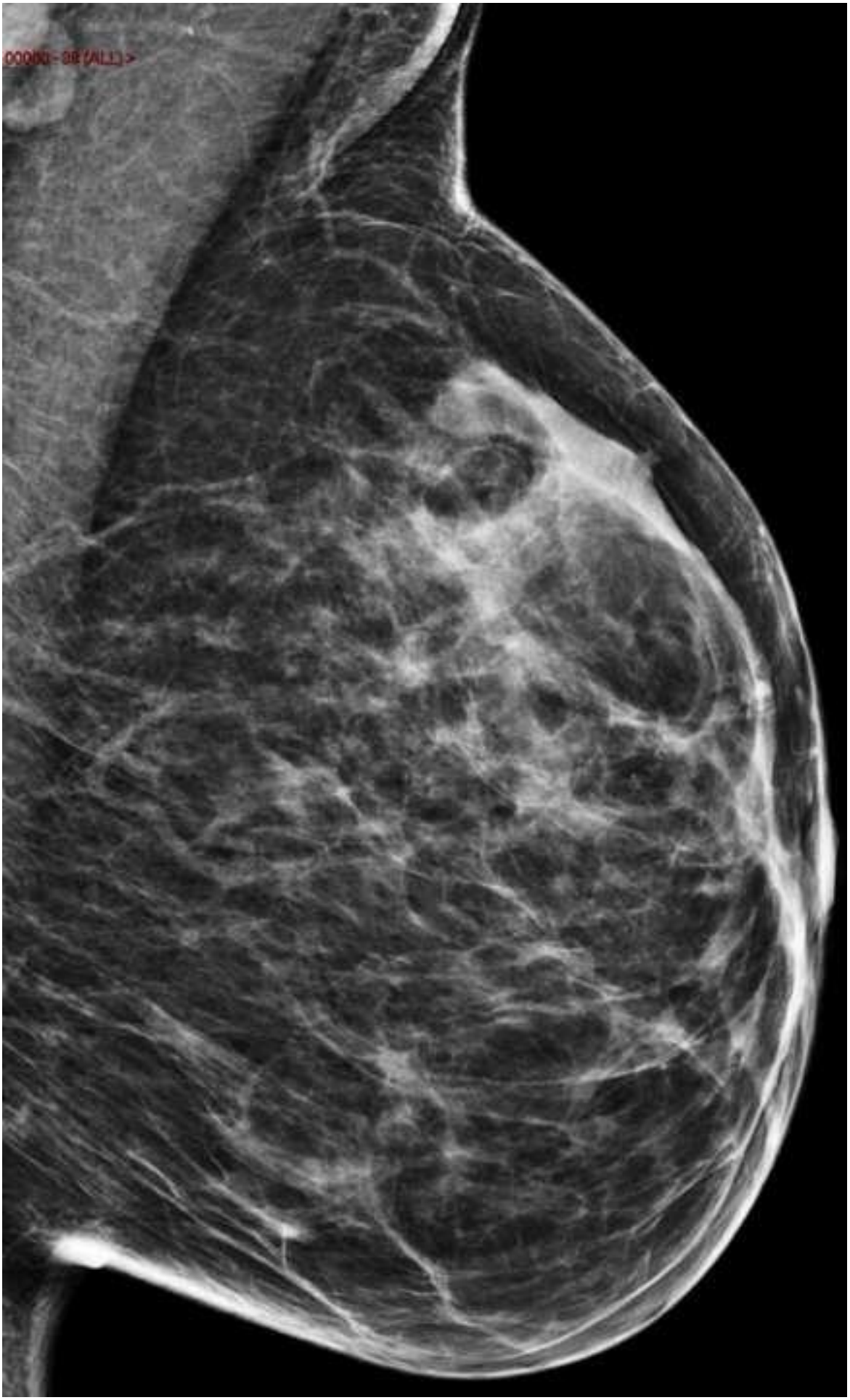

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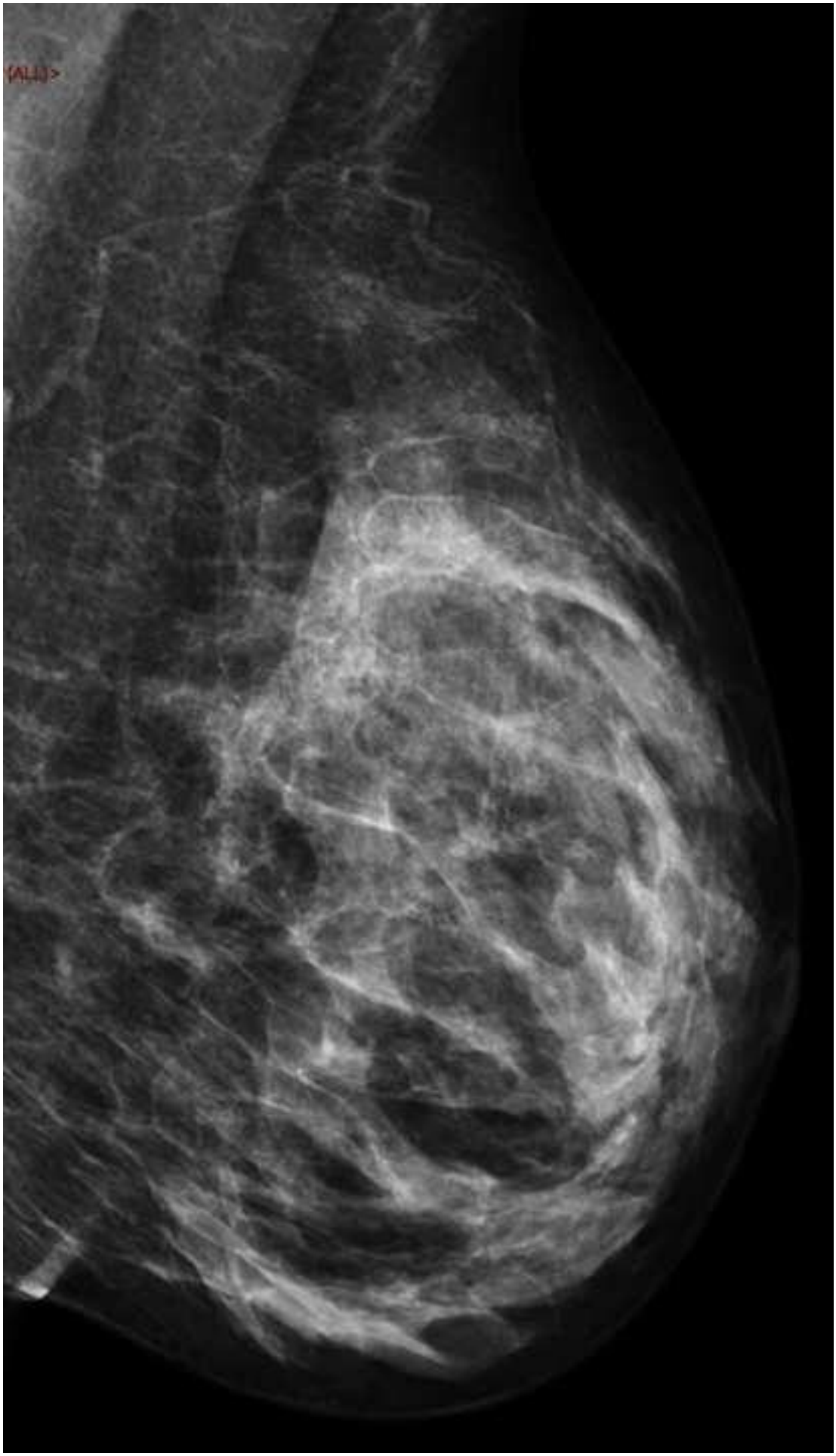

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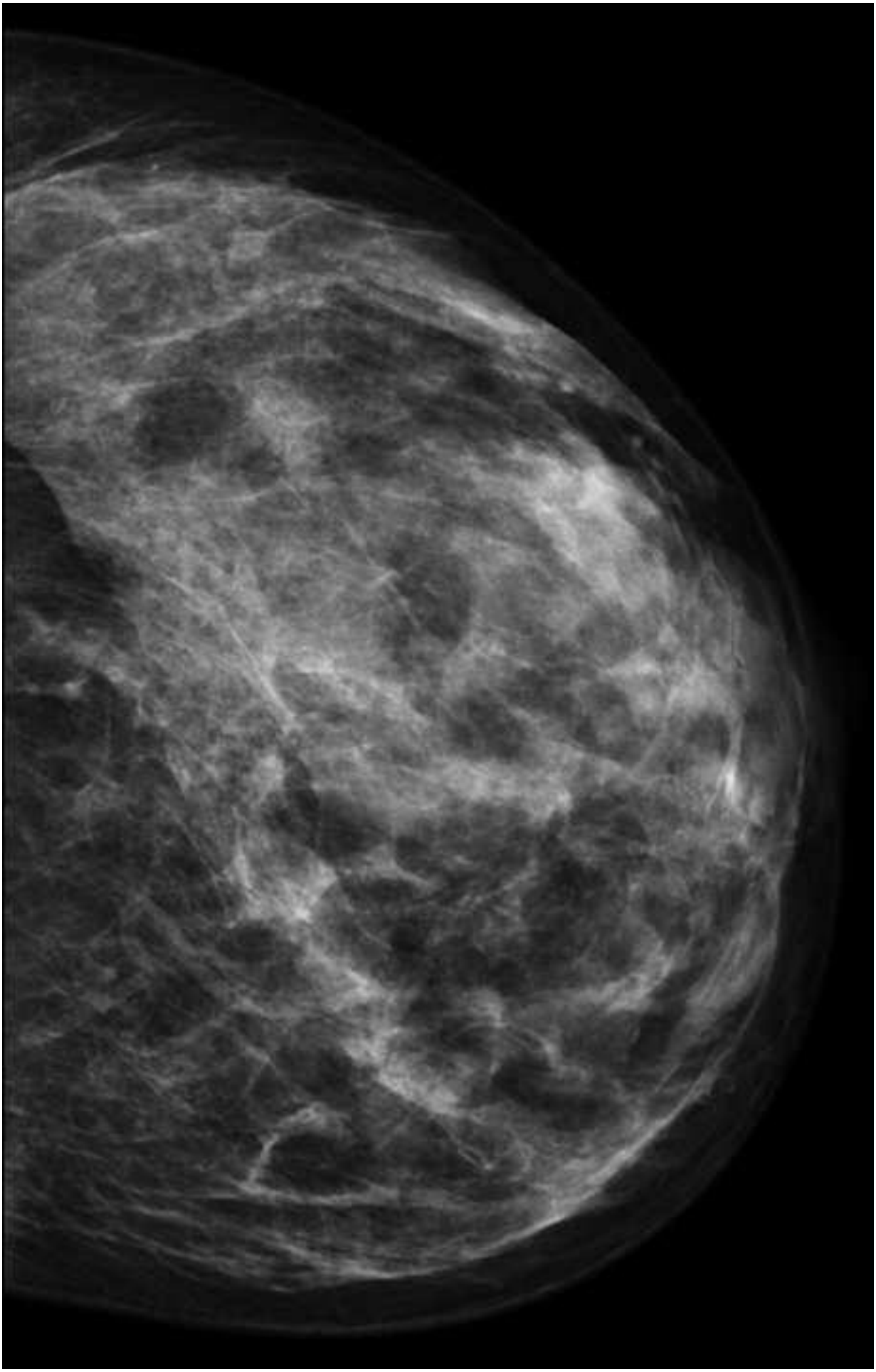

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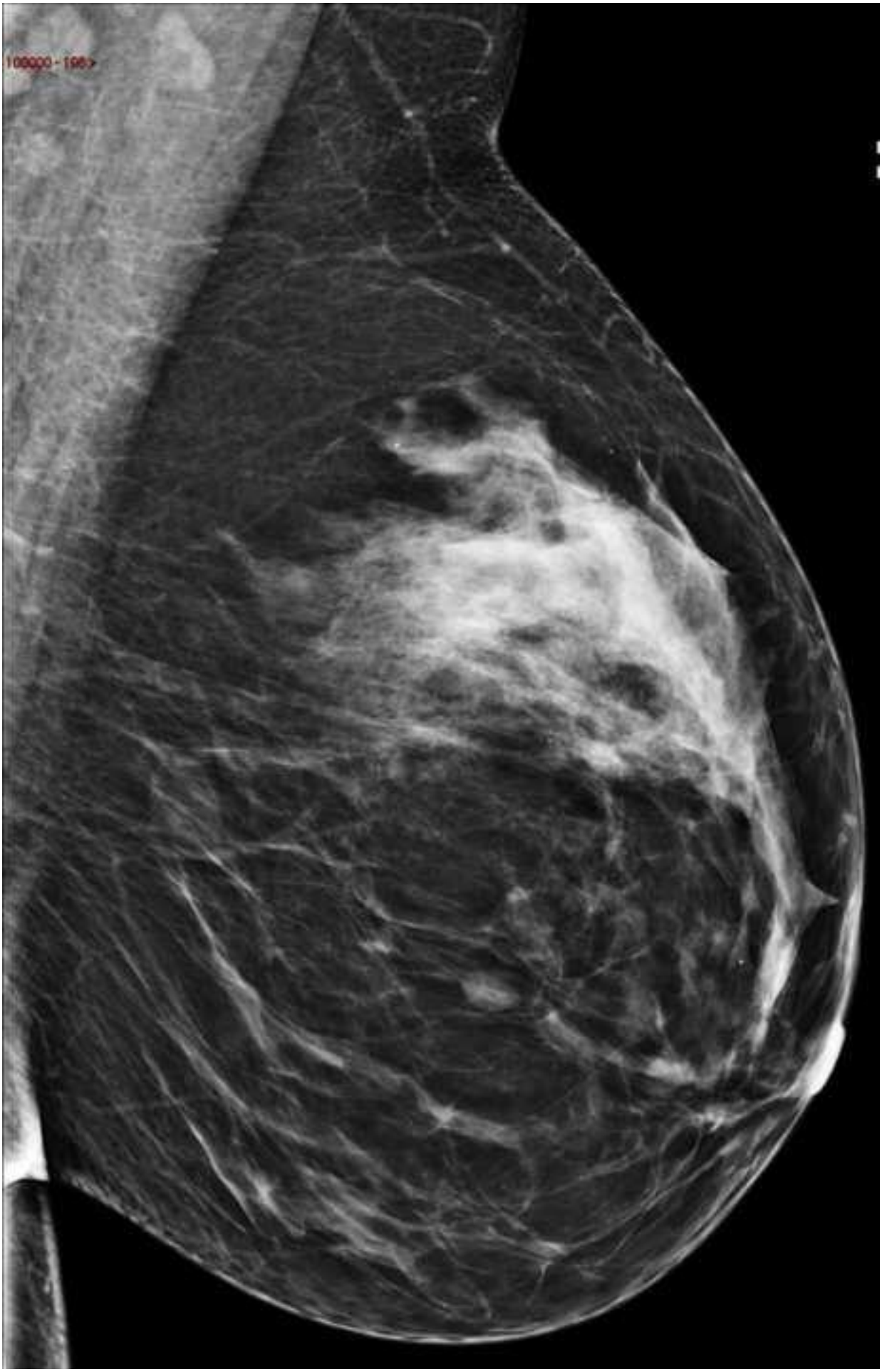

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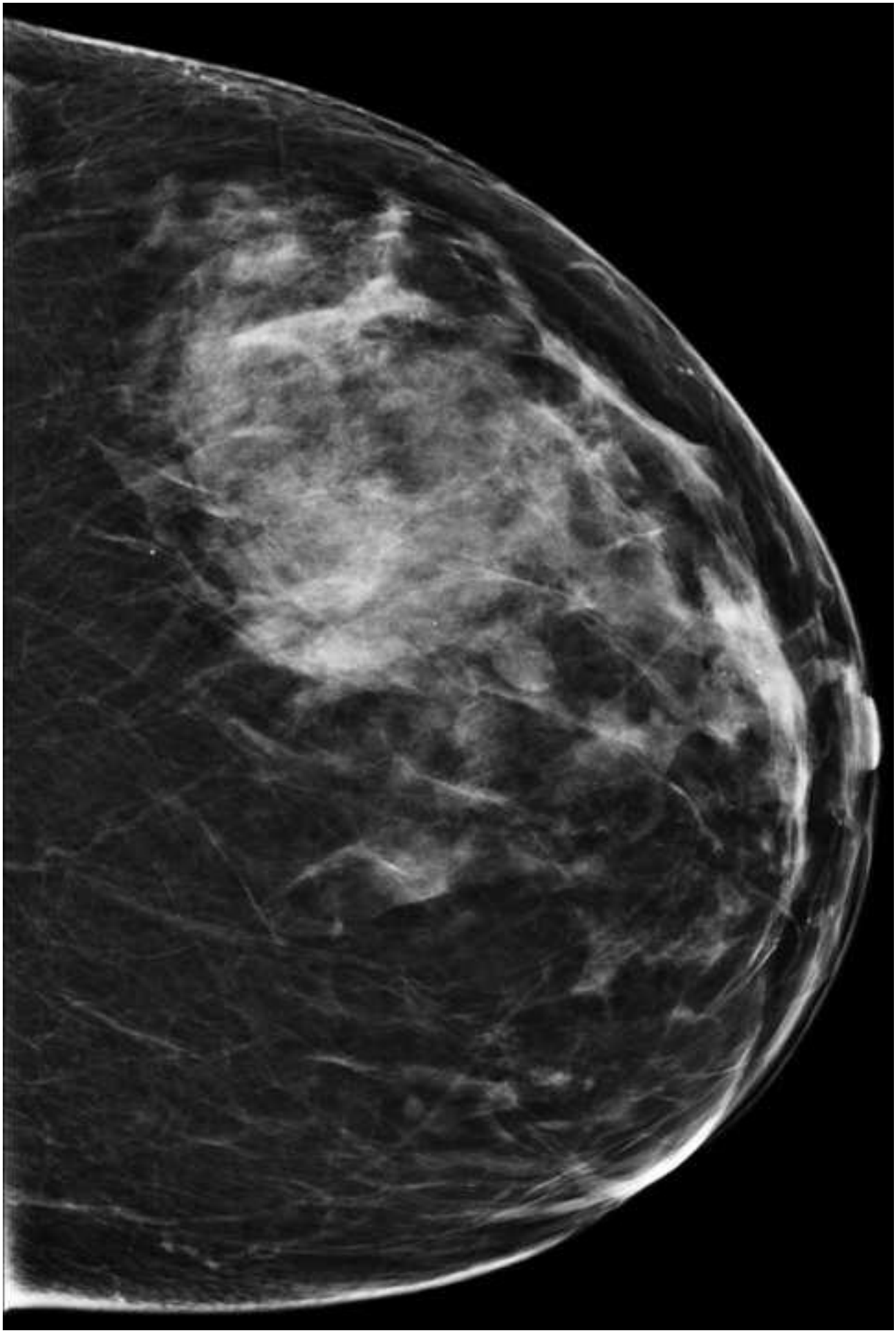

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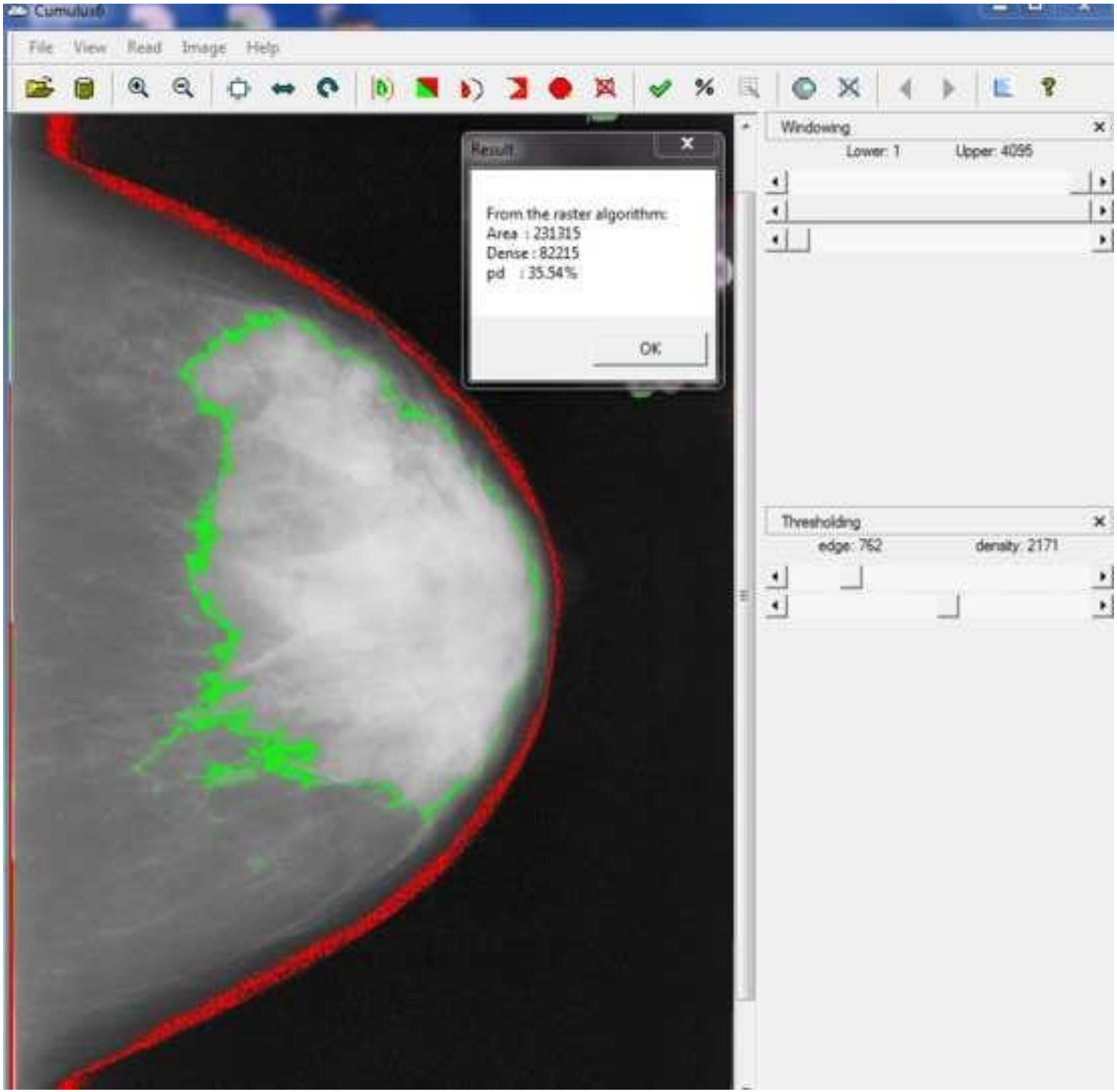
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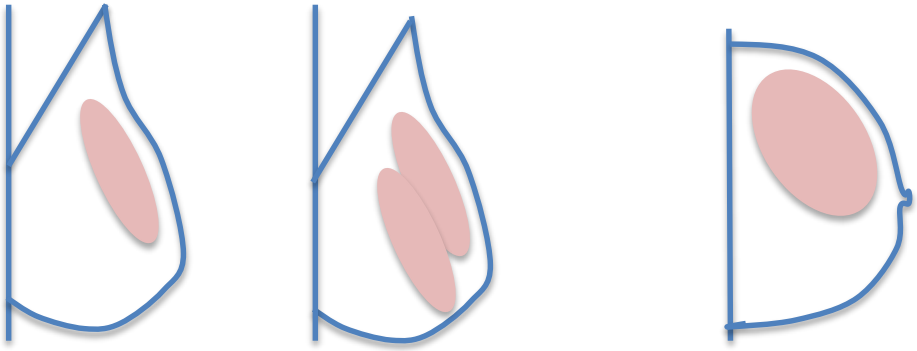

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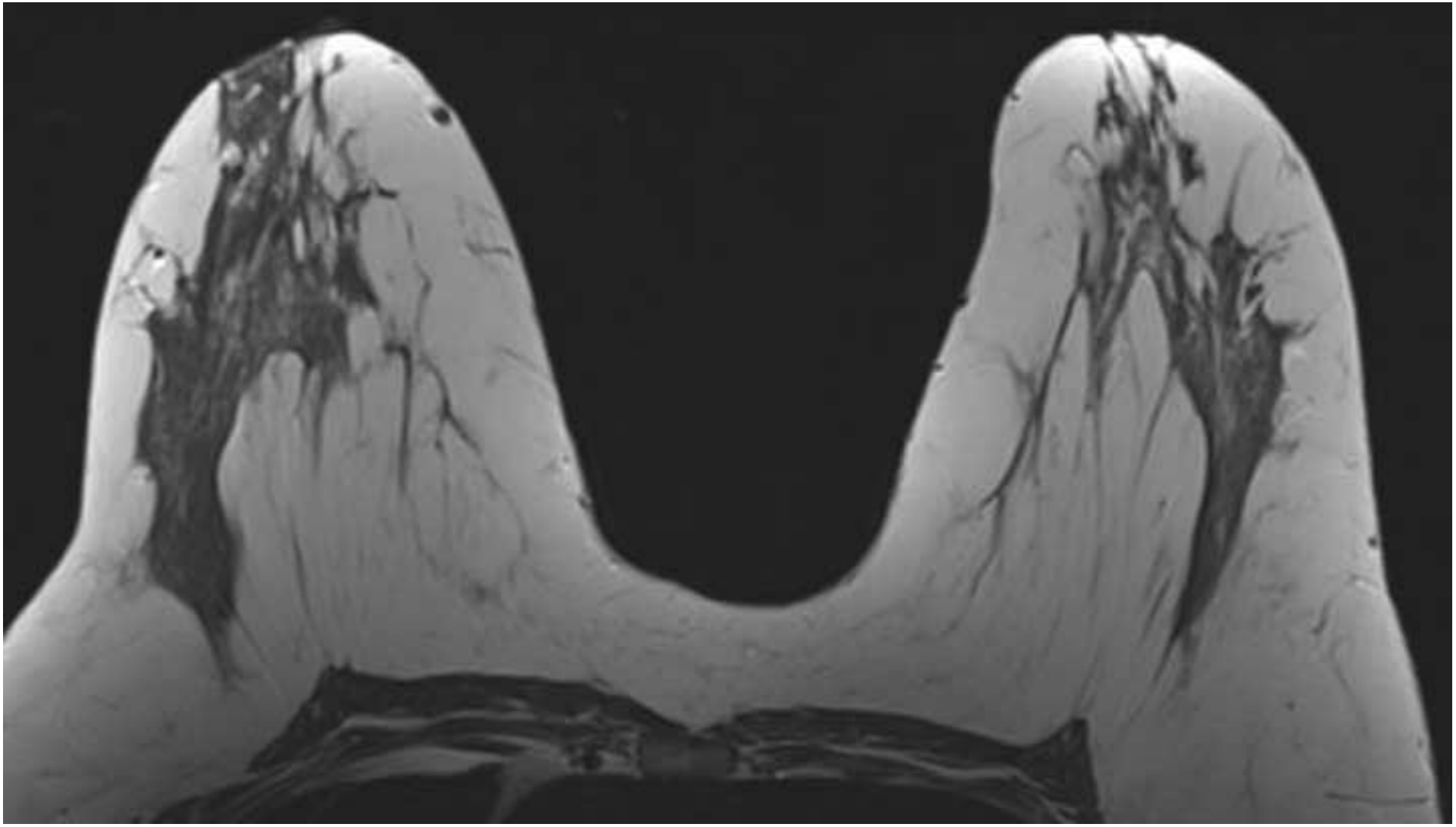

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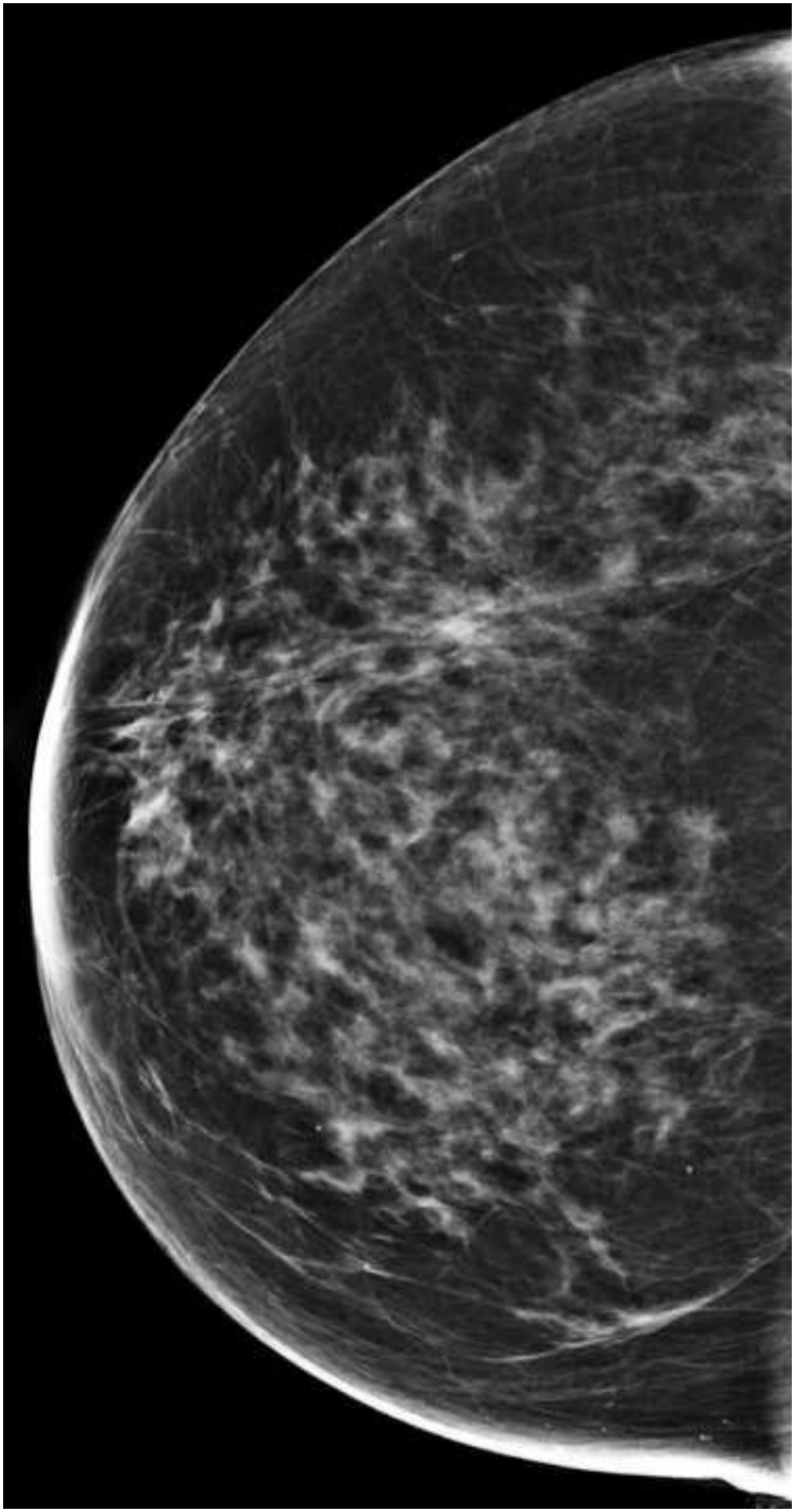

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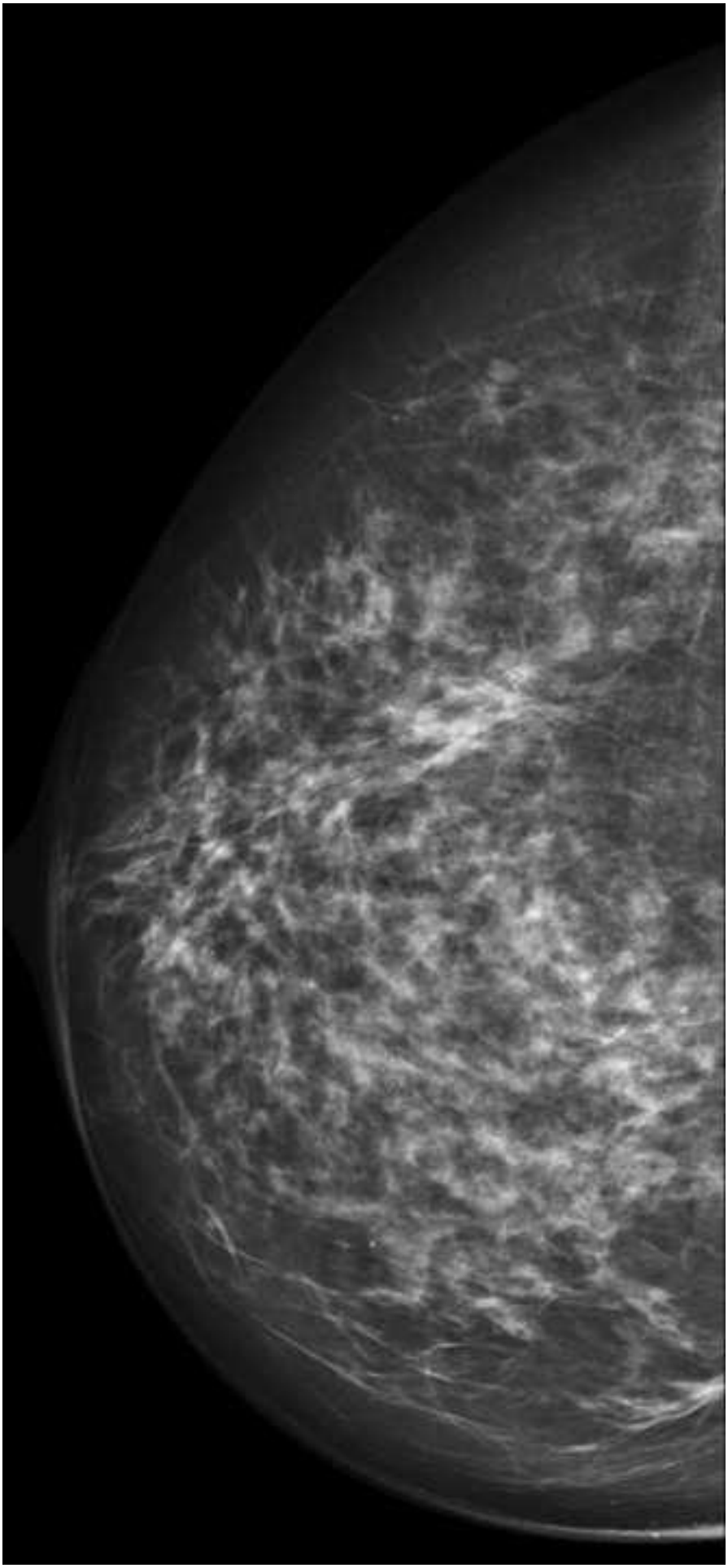

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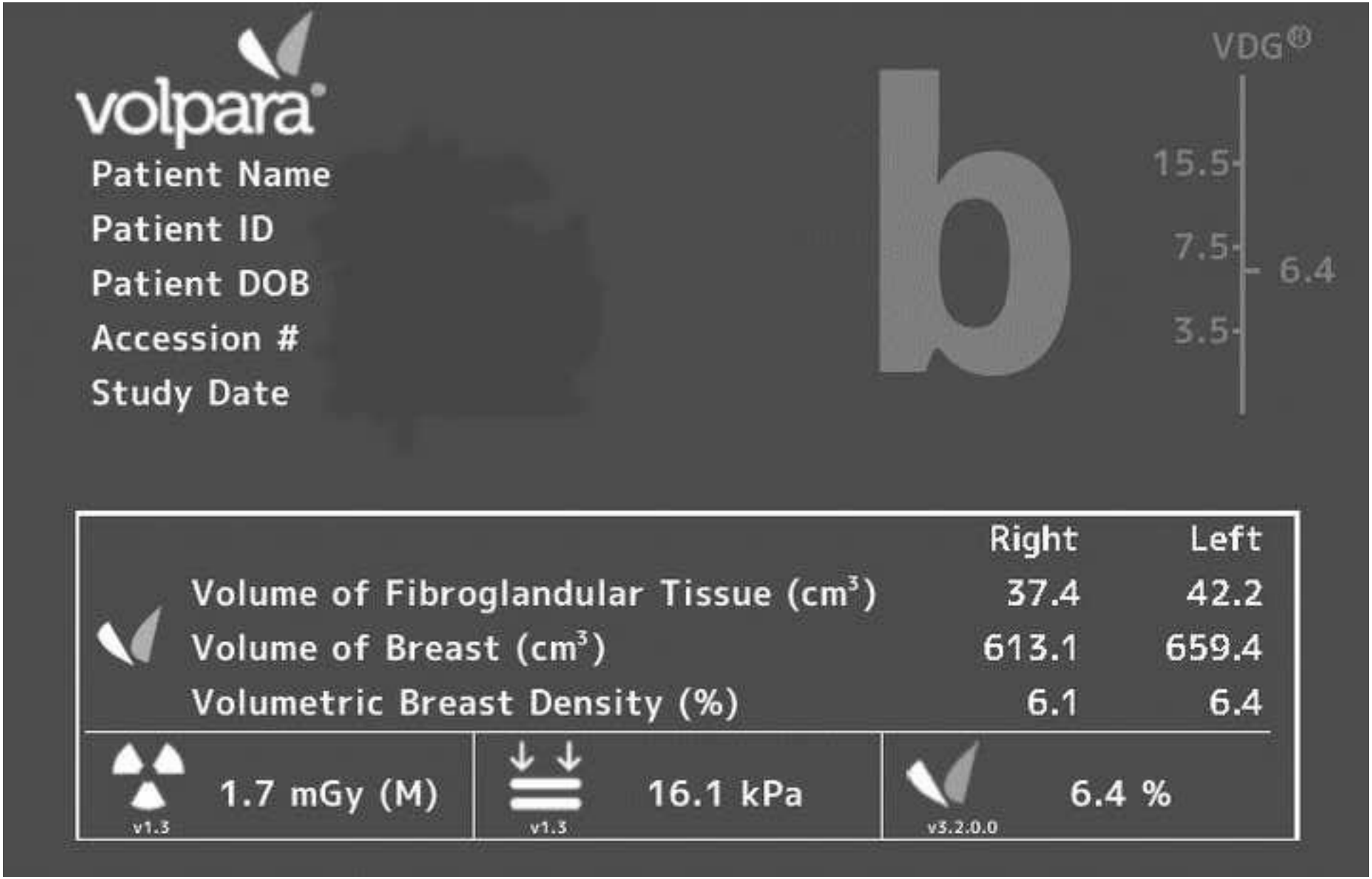

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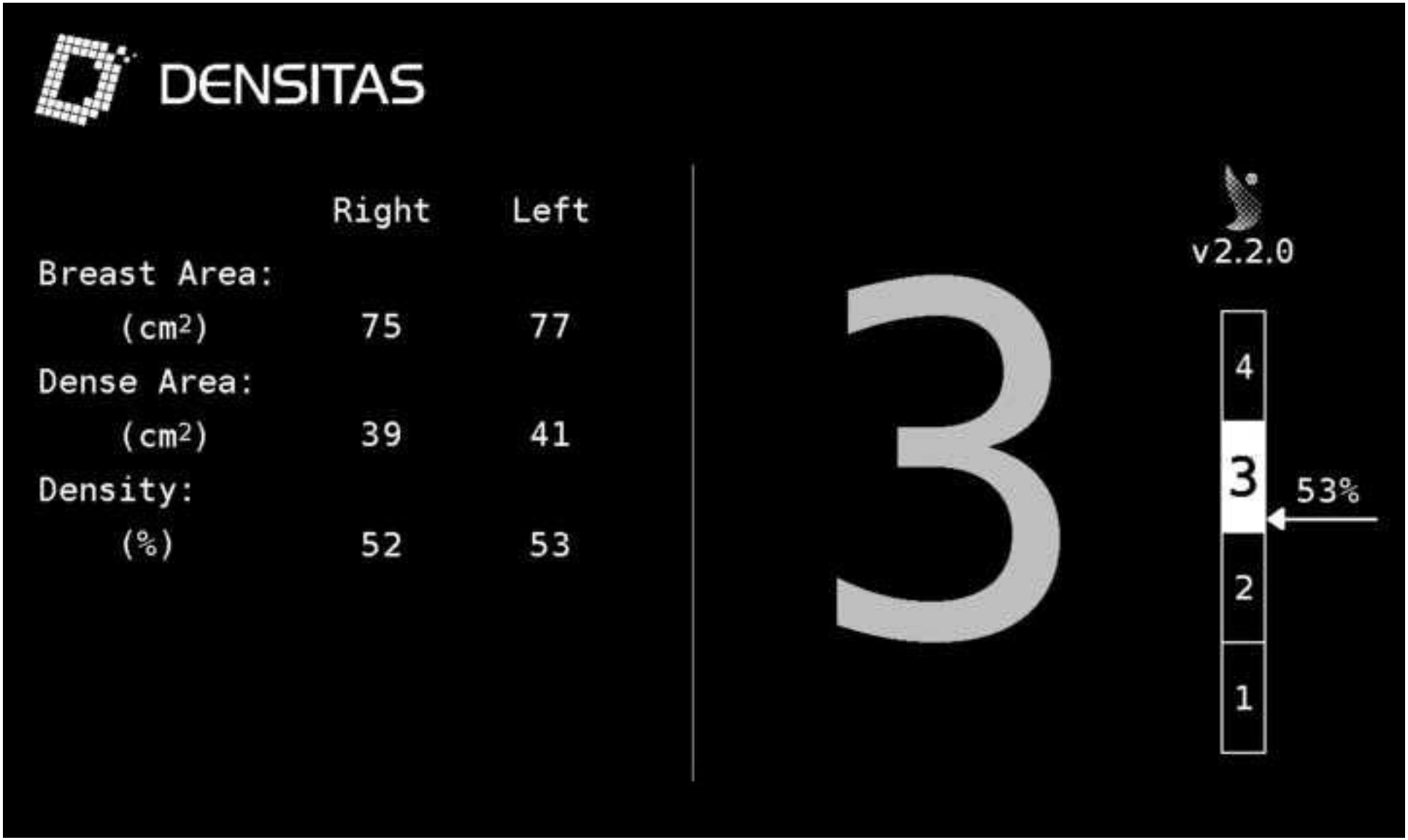


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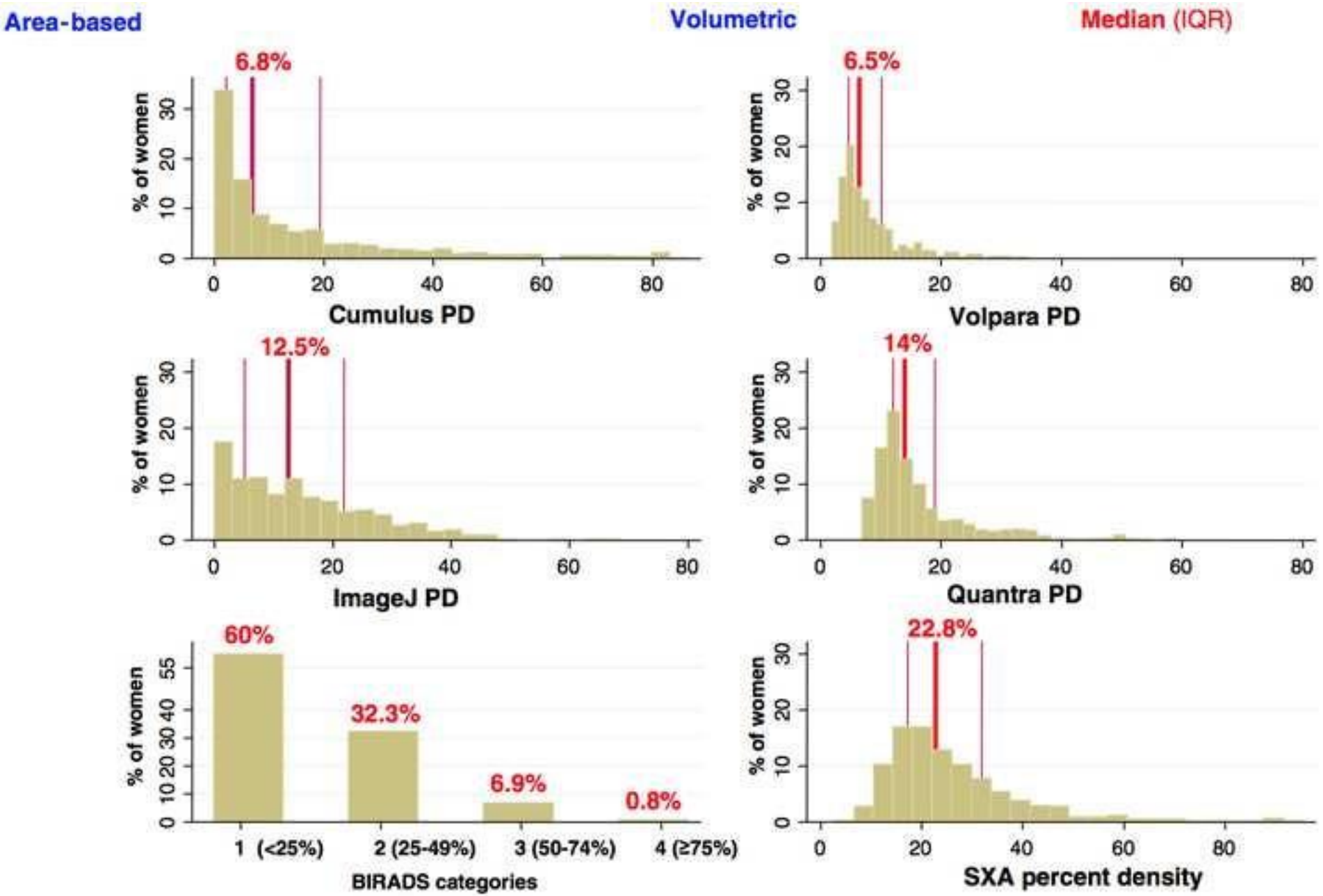


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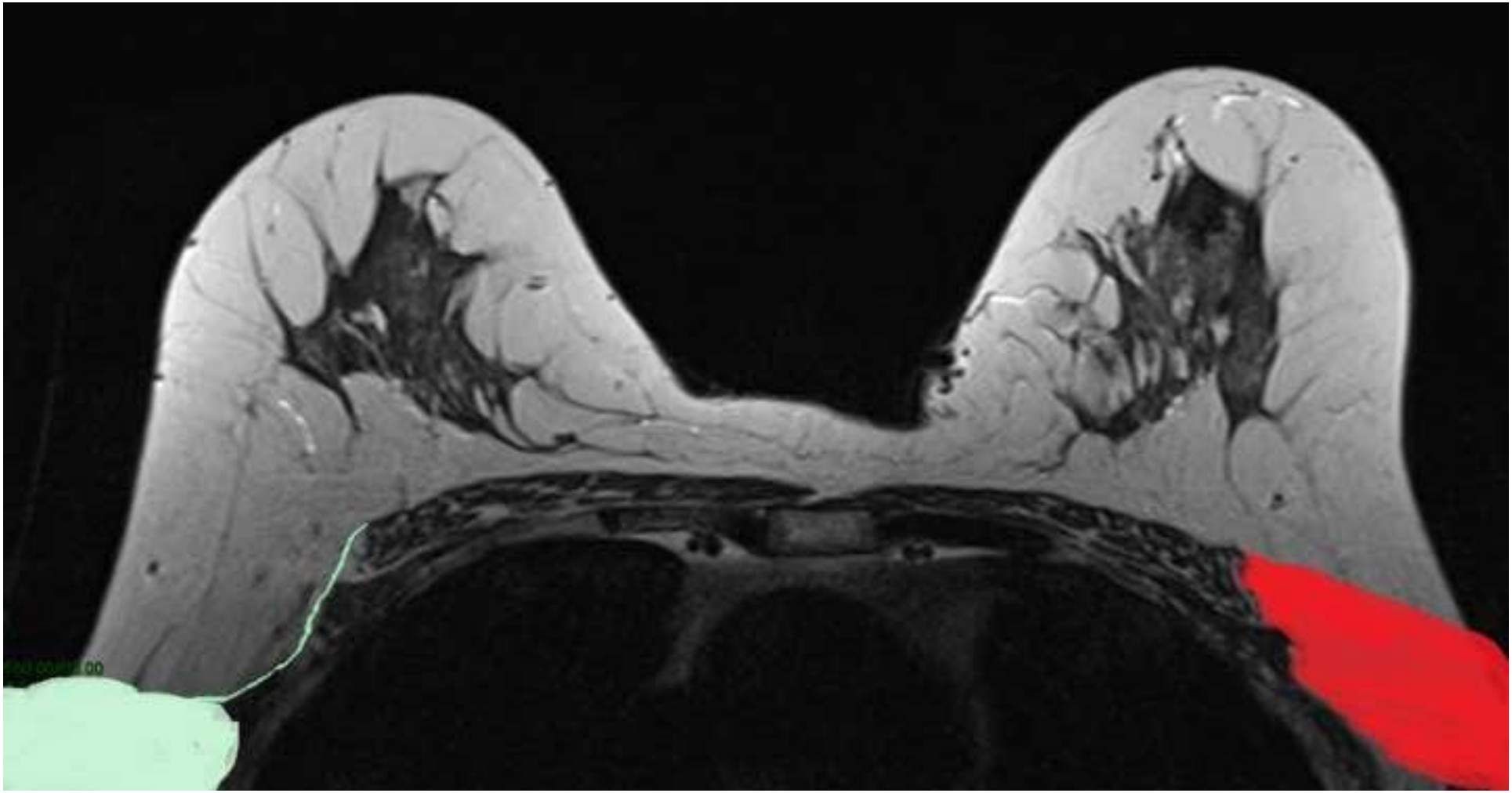

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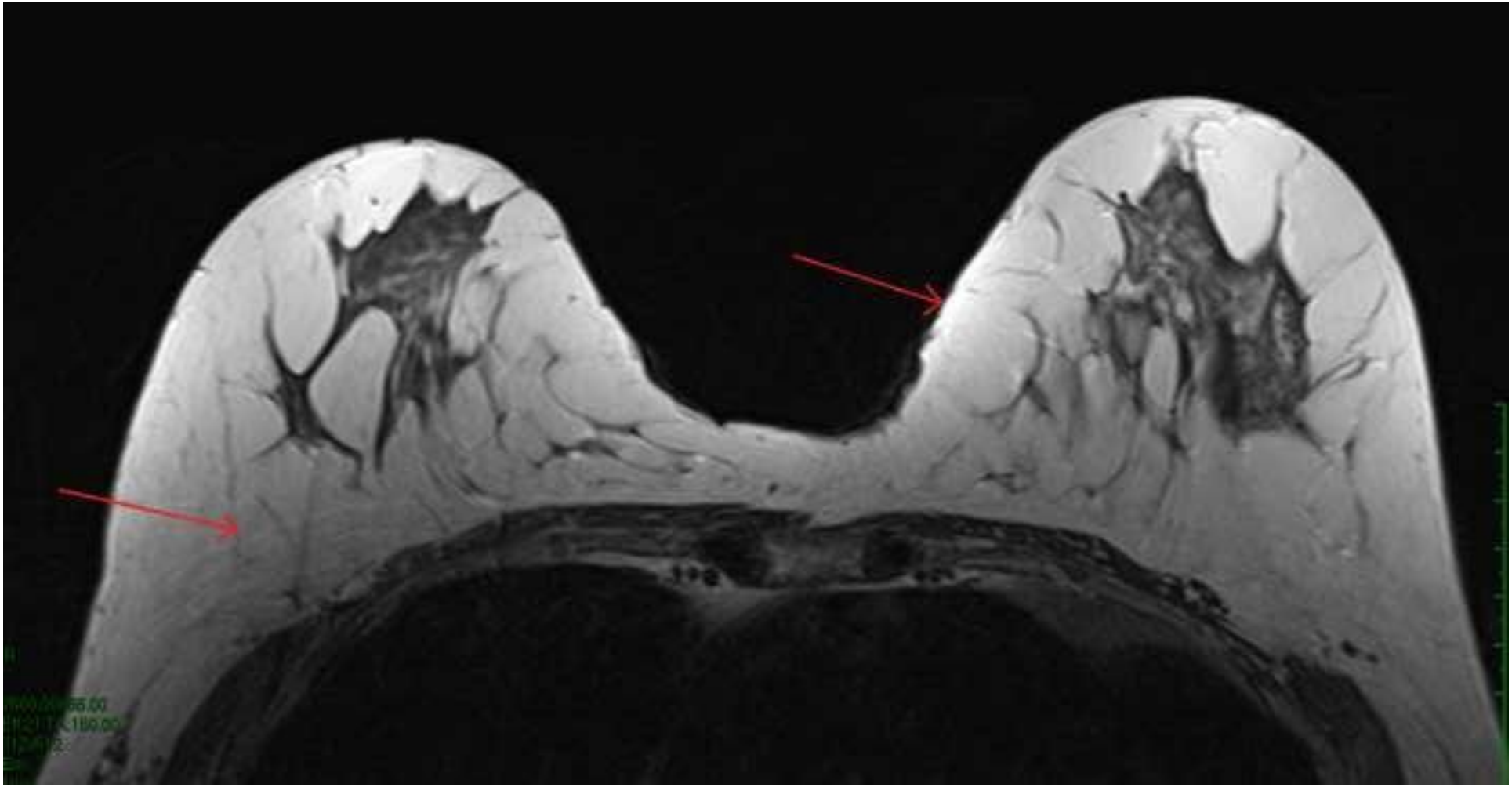

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